Tooth Development III: The Genetic Basis of Inherited Tooth Disorders

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What is the Human Genome Project?

A draft sequence of the entire human genome.

Completed in fewer years than predicted.

Led to the discovery of novel genes and localization of previously known genes.

Overview

- Understand the basic principles of genetics.
- Review the recent advances in craniofacial genetic discovery – specifically tooth agenesis.
- Determine the relationship between dentistry and genetic discovery.
mortal : brick :: glue ::
1. stone
2. wood
3. water
4. oil

Humans share 99.9% of their DNA

The Craniofacial Complex
... as the window to our genes

Of the many genetic syndromes that are characterized in the literature, about 75% have a craniofacial component
Congenitally Missing Teeth

the most common craniofacial anomaly in man

Class III malocclusion is the most common craniofacial anomaly known in man.

1. True
2. False

Jaw Size and Shape

Perception of Pain

• Recent studies at UNC SOD have revealed that certain ‘haplotypes’ are predisposed to dental pain.

External Apical Root Resorption

How Do We Study Genetics?

DNA the molecule of life

Trillions of cells
Each cell:
• 46 human chromosomes
• 3 meters of DNA
• 3 billion DNA subunits (the bases: A, T, C, G)
• Approximately 30,000 genes code for proteins that perform most life functions
**Types of Genetic Diseases**

- **Chromosomal**
- **Single gene Disorders: Mendelian**
- **Multifactorial**
  - Multiple genes
  - Environment

**Single-gene Disorders**

- Gene
- Environment

**The Gene**

The basic unit of inheritance.

- The functional and physical unit of heredity. Genes are pieces of DNA, and most genes contain the information for making a specific protein.

**The Hunt for the Gene**

Glazier et al., 2002, Science 298: 2345-9

**Genetic Versus Environmental Causes for Diseases**

<table>
<thead>
<tr>
<th>Genetic Causes</th>
<th>Environmental Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single Gene Disorders</td>
<td>HTN, B/Ca, CAD, Schizophrenia, Alcoholism</td>
</tr>
<tr>
<td>CF, MFS</td>
<td>Infection, Trauma</td>
</tr>
<tr>
<td>Br/Ca, CAD</td>
<td>DVT</td>
</tr>
</tbody>
</table>

(A) Two pairs of 23 chromosomes are contained within a single cell. (B) Each chromosome represents the basic unit of inheritance.

1. A is true and B is true.
2. A is true and B is false.
3. A is false and B is true.
4. A is false and B is false.
Mutation
A permanent structural alteration in DNA that is usually associated with a harmful effect to the organism. Some mutations can provide a benefit to survival which is passed on to its descendants.

The sun was hot but the old man did not get his hat.

Allele
The sequence of the DNA at a locus; refers to the sequence on a single chromosome.

Single Nucleotide Polymorphisms

A genetic mutation refers to:
1. A common alteration on the DNA sequence.
2. A neutral change in the DNA sequence.
3. An insertion or deletion of a nucleotide.
4. All of the above.

Genetic Variation

Mutation
- Rare change in the DNA sequence
- Refers to a negative effect

Polymorphism
- 2 or more alleles in the population
- May be benign or predispose to disease
- Present in 1% of the population

How do we communicate about genetic information?

Genotype - specific alleles present in an individual at a given locus
**Genetic Communication**

**Heterozygote** - individual with one copy of a particular allele; may also refer to an individual with two different alleles at a locus

This individual is a heterozygote at loci A, B, D, and E

**Homozygote** - individual with two copies of the same allele at a locus; may also refer to an individual with two mutant alleles at a locus

This individual is a homozygote at locus F

**Phenotype** - the observed characteristics of an individual, determined by the genotype and the environment

Disorder X and Disorder Y have nearly identical phenotypes but result from mutations in different genes. This is an example of:

1. Autosomal Dominant
2. Autosomal recessive
3. Variable expressivity
4. Allelic heterogeneity
5. Locus heterogeneity

**Gene-trait Relationship: Complex Disorders**

**Primary Methods for Assessing Genetic Basis of a Disease**

1. Familial aggregation
2. Twin studies
3. Ethnic differences in prevalence
4. Co-segregation with other genetic syndromes
5. Animal models
Animal Models

When a human disease occurs naturally in an experimental system (e.g., mouse), it gives additional support for genetic basis of the trait.

Approaches to Identifying Disease Susceptibility Genes

- Linkage analysis
  - Studies families with more than one affected individual
- Candidate Gene Analysis (Association Study)
  - Case-control of unrelated individuals
  - Transmission disequilibrium of families

Instructions providing all of the information for a living organism to grow and live reside in the cytoplasm.

1. True
2. False

Finding Disease Genes

Nucleus → Chromosome → Band → Gene

Mutation

Genetic Markers

- Tools to find disease genes
- Flags with known locations in the genome
- SNPs - Single Nucleotide Polymorphisms
- VNTR - Variable number of tandem repeats

 Millions of polymorphic loci have been discovered in the human genome
Loci located close together on the same chromosome are linked.

In gene mapping studies, we seek loci (markers) that are linked to a disease-causing mutation.

An allele represents one copy of an individual's genetic makeup at a given locus.

A pair of alleles that are identical are referred to as homozygous.

Single Gene Disorders

- Family Ascertainment
- Linkage Mapping
- Candidate Gene Identification
- Mutational Analysis

Family Ascertainment

Medical and Dental Histories, Examination, Records
Linkage Mapping

Pedigree construction, segregation analysis, Genome-wide scan with polymorphic microsatellite markers

Linkage Analysis

- Recombination – consider parental chromosomes at meiosis

Linkage analysis assesses the frequency of recombination directly in families to infer relative distances between loci

Linkage Analysis

Linkage: the co-segregation of trait with a genetic marker

Does the pedigree below show segregation of the trait with the genetic marker?

1. Yes
2. No

50%

LOD score = likelihood ratio that the marker is linked to the disease

LOD = 3.0; significant evidence of linkage, 1,000:1 odds

LOD = -2.0; significant evidence of non-linkage

Candidate Gene Testing

Look for specific genes in GenBank that are specific to the disorder that you are studying
Mutational Analysis
Sequence analysis and mutation screening of other individuals

Direct sequencing of PCR Products

Ehlers-Danlos Syndrome

- Hyperelasticity of skin
- Skin fragility, joint laxity, ligamentous shortening.

Oral Manifestations

- Hypoplastic enamel
- Periodontal disease at a young age
- Malformed, stunted roots
- Large pulp stones
### Ehlers-Danlos Syndrome

<table>
<thead>
<tr>
<th>Inheritance</th>
<th>Autosomal Dominant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal Features</td>
<td>Ligamentous shortening</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Aortic Aneurysms</td>
</tr>
<tr>
<td>Ocular</td>
<td>Mitral valve prolapse</td>
</tr>
<tr>
<td>Prevalence</td>
<td>1 in 5,000 - 1 in 50,000</td>
</tr>
<tr>
<td>Defective Gene</td>
<td>several collagen genes</td>
</tr>
<tr>
<td>Chromosome</td>
<td>15q15-21.3</td>
</tr>
</tbody>
</table>

### Papillon-LeFevre Syndrome

<table>
<thead>
<tr>
<th>Inheritance</th>
<th>Autosomal Recessive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dermatological</td>
<td>Skin lesions</td>
</tr>
<tr>
<td>Prevalence</td>
<td>Very rare</td>
</tr>
<tr>
<td>Defective Gene</td>
<td>Cathepsin C</td>
</tr>
<tr>
<td>Chromosome</td>
<td>11q14.1 - q14.3</td>
</tr>
</tbody>
</table>

### Complex Disorders

- **Heritability?**
  - e.g. twin studies

  - Linkage Mapping
  - Association Analysis

### Orthodontics

- Tooth Agenesis

### Patterning of Human Dentition

- Cleidocranial dysplasia
Previous studies in mice and other vertebrates have implicated several homeobox proteins in tooth organogenesis. (Sharpe, 1995)

The homeobox is a conserved 180 base pairs DNA motif found in eukaryotes and is linked with developmental regulation. (Lewin, 2000)

Regulation of Tooth Development

Tooth Agenesis

“Delineating the phenotype”

- Hypodontia - one or more congenitally missing teeth
- Oligodontia - six or more congenitally missing teeth
  Molar oligodontia - refers to selective agenesis of predominantly posterior teeth with molars being the most affected
- Total anodontia - usually associated with ED

How BIG is the problem of tooth agenesis?

- Esthetic
  - Loss of vertical dimension,
  - Knife-edge alveolar ridge
  - Drifting of teeth, diastemas
  - Complex treatment plan
- Psychosocial consequences
- Financial burden
  - “Posterior tooth agenesis may cost up to $50,000 in out of pocket costs.”
The previous slide showed a pedigree segregating for tooth agenesis. What mode of inheritance is observed?

1. mitochondrial inheritance
2. X-linked dominant
3. autosomal dominant
4. autosomal recessive
5. X-linked recessive
Diagnosis: Non-Syndromic Oligodontia
(18 Congenitally Missing Permanent Teeth)

Family #1

Linkage Mapping
Pedigree construction, segregation analysis, Genome-wide scan with polymorphic microsatellite markers

A) Ehlers-Danlos syndrome is an autosomal recessive disorder; B) this pattern of inheritance often does not ‘skip’ a generation.

1. A True; B True
2. A True; B False
3. A False; B True
4. A False; B False

Linkage Analysis
Linkage: the co-segregation of trait with a genetic marker

Tooth Agenesis
Candidate Gene Testing

Look for specific genes in GenBank that are specific to the disorder that you are studying.

Pax9 Mutants Arrest at the Bud Stage in Mice

E13.5

E14.5

Mutational Analysis

Sequence analysis and mutation screening of other individuals.

PCR Amplification

Analysis of Human PAX9

Frameshift Mutation Creates an Altered and Truncated Protein

Guanine insertion in the paired domain
Different point mutations are associated with patterns of tooth agenesis that are remarkably similar (posterior teeth).

Loss of function most likely mechanism.

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**Tooth Agenesis**

<table>
<thead>
<tr>
<th>Gene</th>
<th>OMIM #</th>
<th>Phenotype</th>
<th>Genotype (mutation)</th>
<th>Pattern of tooth agenesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSX1</td>
<td>106600</td>
<td>Hypodontia</td>
<td>Exon 2 (HD); Vastardis et al., 1996; Lidral and Reising, 2002</td>
<td>Mostly premolars, rare incisors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exon 1; Van den Boogaard et al., 2000</td>
<td>Mostly premolars</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mostly tooth agenesis; Liu et al., 2001</td>
<td>Mostly tooth agenesis</td>
</tr>
<tr>
<td>PAX9</td>
<td>604625</td>
<td>Non-syndromic tooth agenesis</td>
<td>Exons 2 and 4; Stockton et al., 2000; Nieminen et al., 2002; Fraziers-Bowers et al., 2002</td>
<td>Mostly molars and premolars</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exons 1; Fraziers-Bowers et al., 2003</td>
<td>Mostly molars and premolars, rare incisors</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exon 2 (HD); Jumlongras et al., 2001</td>
<td>Mostly premolars and first molar</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Several; The Intl. Incontinentia Pigmenti Consortium, 2000</td>
<td>Skin, hair, and oral abnormalities</td>
</tr>
</tbody>
</table>

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**Incontinentia Pigmenti**

- Disturbance of skin pigmentation with malformations of:
  - Teeth
  - Skeleton
  - CNS

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**Reiger's Syndrome**

- Autosomal Dominant Inheritance with complete penetrance and variable expressivity
- Clinical manifestations include dental, ocular, and other abnormalities
- Mutation in Pitx2/Reig gene causes this condition
Reiger’s Syndrome

- Partial or complete anodontia (oligodontia)
- Most commonly missing primary and permanent maxillary incisors
- Microdontia—typically small cone shaped anterior teeth
- Relative mandibular prognathism due to hypoplastic maxilla

Reiger’s Syndrome

Witkop’s Tooth and Nail Syndrome

- Usually autosomal dominant
- Characterized by nail dysgenesis, missing and malformed teeth
- Jumlongras et al (2001) discovered that a mutation in MSX2 caused Witkop’s TNS

Ectodermal Dysplasia

- Hypohidrotic ectodermal dysplasia belongs to a heterogeneous group of disorders of the ectodermal tissue.
- Over 120 different types of ectodermal dysplasia have been reported. They are characterized by a number of defects involving the teeth, skin, and appendageal structures (hair, nails, and eccrine and sebaceous glands)
- Generally, this syndrome is an X-linked recessive disorder caused by EDA1 gene within the region Xq12-q13.1 (Ferguson 1998, Zonana 1992-1993), and characterized by a triad of defects including: Hypohidrosis, Hypotrichosis and Anomalous Dentition
**Ectodermal Dysplasia**

- **Hair:**
  - Hypotrichosis
  - Alopecia
  - Fine, dry, silky, hypochromic hair

A 4-year old HED girl with sparse and fragile hair

A 13-month old HED boy lacking hair, eyebrows, eyelashes and teeth

- **Craniofacial features:**
  - Saddle-nose: low nasal bridge, small nose with hypoplastic alae nasi
  - Frontal bossing
  - Prominent supraorbital ridges
  - Prominent lips due to reduced lower facial height
  - Periorbital pigmentation
  - Small palatal and cranial base widths

- **Teeth:**
  - Absent teeth (hypodontia to anodontia)
  - Small pointed conical incisors

The genes most frequently known to be causative of non-syndromic tooth agenesis include:

1. **EDA1 and MSX1**
2. **MSX1 and PAX9**
3. **PAX9 and EDA1**
4. **MSX1 And PITX2**
5. **PAX9 and PITX2**
Van Der Woude Syndrome

- Syndrome occurring in 2% of patients with facial clefts
- Classified as autosomal dominant with variable expressivity
- Mutations in \( IRF6 \) (interferon receptor regulatory factor) responsible for most cases

Van der Woude syndrome

- Van der Woude syndrome patients have congenital sinuses of the lower lip or cleft palate, or cleft lip, or any combination of the three symptoms
  - Most common cause of syndromic cleft cases
  - Affects 1:100,000-200,000 people
  - Accounts for 2% of all cleft lip and palate cases
  - No gender or racial preference
  - Patients exhibit normal intelligence

Variable expressivity

- 25% of pts with VWS have no or minimal findings, such as:
  - Absent teeth
  - Lip pits
- Pts may present with only lip pits, lip pits with cleft palate or only cleft lip and/or palate
- Clefts of lip and palate in 21%
- Occasional reports of hypodontia and sygnathia

Clinical Findings

- Lip pits:
  - Present in 68% of affected
  - Only manifestation in 64%
  - Blind sinuses, communicate with minor salivary gland
  - Often transport viscid saliva, causing discharge

Clinical Findings

- Cleft of the lip and/or palate:

Clinical Findings - Hypodontia

- Many references site this as a feature of VWS
- This, also, may be the only manifestation of the syndrome
- Gorlin syndrome text:
  - Seen in 10-20% of VWS gene carriers
  - Absence of maxillary and mandibular premolars
  - Ranta and Rintala, Angle Orthod, 1982; Schneider, J Med Genet 10, 1973
**Clinical Findings**

- **Sygnathia:**
  - Epithelial strands running from the maxilla to the mandible
  - Impede mouth opening

- More commonly seen in popliteal pterygium syndrome
- Over two-fifths of patients exhibit this anomaly

**Van Der Woude Syndrome**

**Differential Diagnosis**

- PPS can be differentiated from VWS by the popliteal web in most cases
  - Mildly affected PPS pts may be impossible to differentiate from VWS patients
  - If the nail anomaly of triangular overgrowth of skin and clefting of lip and/or palate are present, then diagnosis of PPS should be made
- VWS and PPS can be differentiated from isolated clefts by family history
  - This may require closer examination of family members for minimally affected individuals

**Etiology**

- VWS/PP are autosomal dominant syndromes
  - 50% chance of passing gene to child of any sex
- 30-50% of all cases of VWS arise as de novo mutation
- High degree of penetrance (how often symptoms are exhibited in the presence of the gene mutation)
  - 20% chance for child to inherit cleft from an affected parent
  - 25% chance to inherit lip pits only or being nonpenetrant
- Phenotypic overlap and gene linkage data suggest that VWS and PPS are allelic

**Dental Supportive Treatment**

- **Orthodontics:**
  - Palatal expansion often required prior to alveolar graft
    - Major and minor segments of the palate collapse on themselves due to scarring from palatal closure at 12-16 months
  - Expansion:
    - approximates segments
    - narrows the cleft to create a better skeletal base for nasal reconstruction
  - Reverse pull face mask often necessary during comprehensive orthodontics to reduce midface deficiency

**Fig. 21-37. Van der Woude syndrome. (A) Note repugated unilateral cleft lip, asymmetric masses of lower lip. (B) Symmetrical paramedian clefts of lower lip. (C) Photomicrograph of 7.5 mm embryo demonstrating three in registrations in mandibular process. Those disappear by the 14 mm stage. (From JC Wurmbach et al. Br J Plast Surg 4:254, 1952.)**

**Fig. D38. Osteocutaneous syndrome. A. Hypoplastic frenum and palatal clefting. B. Facial chisel and angled root in anterior patient. C. Full lateral views, right hemimandible and hypoplastic bone.**