# Next Generation Sequencing Leads To Genotype/Phenotype Correlations Enlightening Amelogenesis Imperfecta Classification

**Agnès Bloch-Zupan[1,2,3,4,5], Tristan Rey[5,6], Alexandra Jimenez-Armijo[5], Marzena Kawczynski[2,3], O-Rares network [7], Bénédicte Gérard[6], Marie-Cécile Manière[1,2,3,4], Virginie Laugel-Haushalter[1,5,6]**

[1]Université de Strasbourg, Faculté de Chirurgie Dentaire, Strasbourg, France
[2]Hôpitaux Universitaires de Strasbourg (HUS), Pôle de Médecine et Chirurgie Bucco-dentaires Hôpital Civil
[3]Centre de référence des maladies rares orales et dentaires, O-Rares, Filière Santé Maladies rares TETE COU
[4]European Reference Network ERN CRANIO, Strasbourg
[5]Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), INSERM U1258, CNRS- UMR7104, Illkirch, France
[6]Service de Génétique Médicale, Hôpitaux Universitaires de Strasbourg, Institut Génétique Médicale d'Alsace, Strasbourg, France
[7] CRMR de Strasbourg (Pr Agnès Bloch-Zupan; Pr Marie-Cécile Manière; Dr Adeline Loing); CCMR de Nantes (Dr Serena Lopez-Cazaux); CCMR de Lyon (Pr Jean-Jacques Morrier, Dr Jean-Pierre Duprez), CRMR de Paris (Pr Tiphaine Davit-Beal; Pr Benjamin Fournier) ; CCMR de Toulouse (Pr Isabelle Bailleul-Forestier); Faculté de médecine de Rabat, Maroc (Pr Mustapha El Alloussi); Faculté de médecine de Prague, République Tchèque (Pr Tatjana Dostalova)

## Introduction

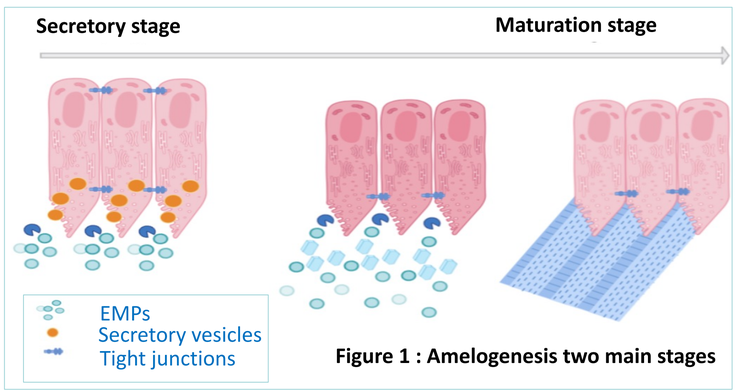



**Figure 1 : Amelogenesis two main stages**

| | | | |
|---|---|---|---|
| Type I hypoplastic | IA | hypoplastic, pitted | AD |
| | IB | hypoplastic, local | AD |
| | IC | hypoplastic, local | AR |
| | ID | hypoplastic, smooth | AD |
| | IE | hypoplastic, smooth | XLD |
| | IF | hypoplastic, rough | AD |
| | IG | enamel agenesis | AR |
| Type II hypomaturation | IIA | hypomature, pigmented | AR |
| | IIB | hypomature | |
| | IIC | snow-capped teeth | XL |
| | IID | hypomature | AD |
| Type III hypocalcification | IIIA | hypocalcified | AD |
| | IIIB | hypocalcified | AR |
| Type IV hypomaturation/hypoplastic with taurodontism | IVA | hypomature/hypoplastic, taurodontism | AD |
| | IVB | hypomature/hypoplastic, taurodontism | AD |

**Figure 2 : Witkop classification**

**Enamel** is the only highly organized and hard mineralized structure from ectodermal origin. It contains 96 to 98% of hydroxyapatite minerals and is neither vascularized nor innervated. It is therefore incapable of regeneration nor reparation.

**Amelogenesis is classically divided in 2 stages.** During the **secretory stage,** ameloblasts are secreting enamel matrix proteins creating the organized scaffold for mineralization. During **maturation stage,** ameloblasts are involved both in enamel matrix protein degradation through enzymes activities, in ions transport and in the maintenance of pH to allow appropriate growth of enamel cristals **(Figure 1).**

AI can be described clinically according to the enamel defect and classified as hypoplastic, hypomineralized or hypomature and serve as a basis, together with the mode of inheritance, to Witkop's classification **(Figure 2).**
AI can be observed in isolation or associated with other symptoms in syndromes

## Methods

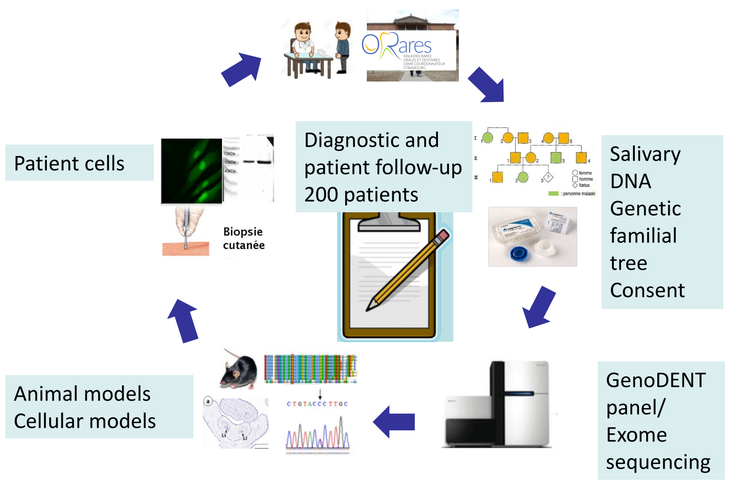



**Figure 3 : Diagnostic and follow-up of patients**

Two hundred patients presenting with so called isolated or syndromic Amelogenesis imperfecta were enrolled and phenotyped at the Reference Centre for Rare Oral and Dental Diseases (O-Rares) using D4/phenodent protocol (www.phenodent.org).

Families gave a written informed consent for the molecular analysis and diagnosis using a dedicated NGS panel called GenoDENT containing 516 genes (Prasad et al., 2016; Rey et al., 2019) **(Figure 3).**

## Results

### 1) One hundred seventeen patients diagnosed (86 isolated, 31 syndromic)

In patient presenting isolated AI, 39 were diagnosed with hypoplastic AI, 28 with hypomature AI, 15 with hypomineralized AI and 3 with hypoplastic AI with taurodontism **(Figure 4).** In patient presenting with syndromic AI, 10 were diagnosed with hypoplastic AI and nephrocalcinosis, 6 with hypoplastic AI and brachyolmia and 4 with Kohlschutter-Tonz syndrome. Other syndromes were diagnosed only in one or two patients **(Figure 5).**

**Figure 4 : Type of isolated AI diagnosis**

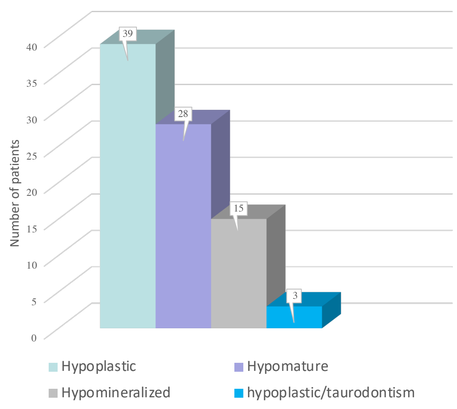
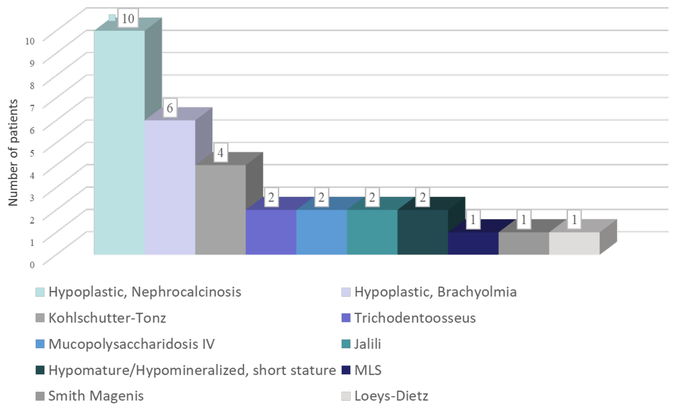



**Figure 5 : Type of syndromic AI diagnosis**




### 2) Variations found in 27 different genes, variations in *MMP20* and *FAM83H* were the most frequent mutations detected by the panel

**Figure 6 : Genetic diagnosis for isolated AI**

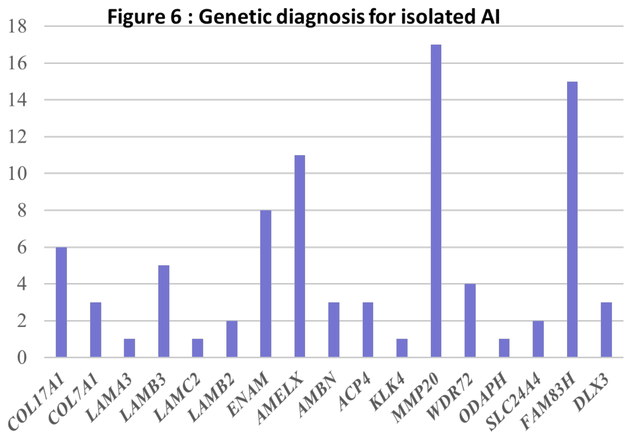
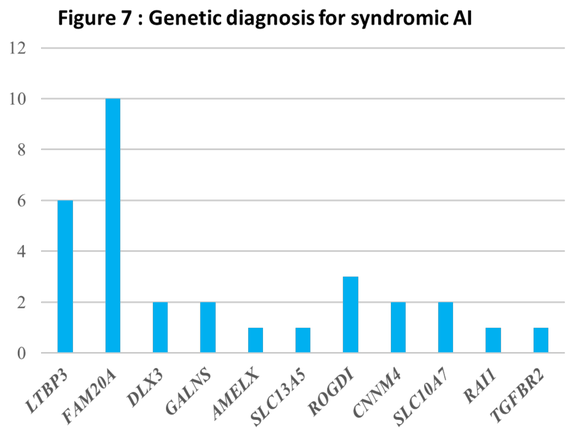
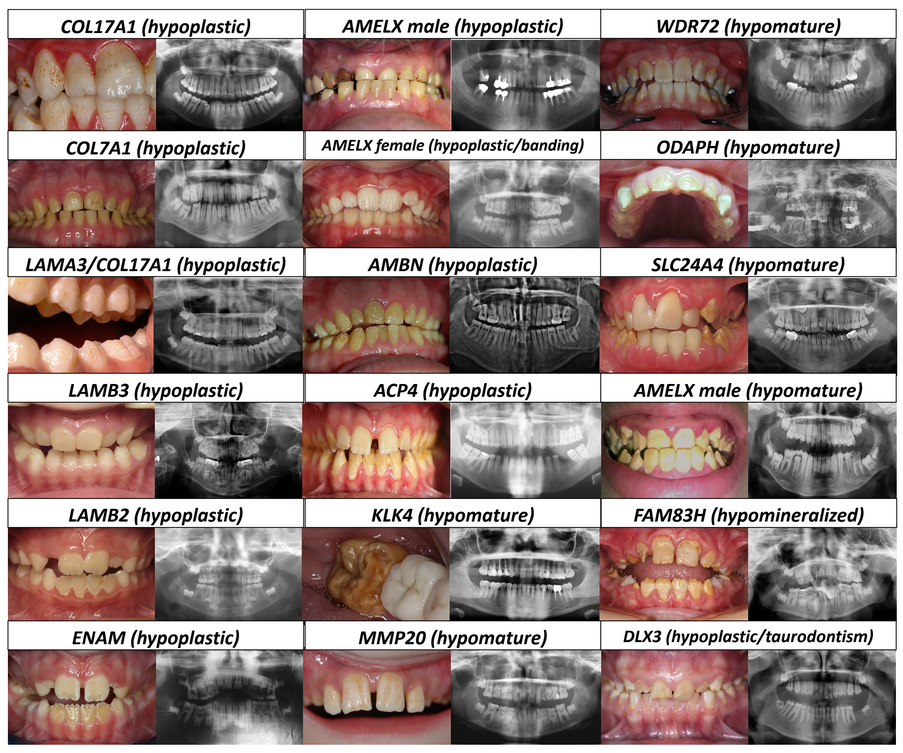



**Figure 7 : Genetic diagnosis for syndromic AI**




In patients presenting isolated AI, the most frequent mutations detected by the panel were found in the *MMP20* gene **(Figure 6).**
In patients presenting syndromic AI, the most frequent mutations detected were found in the *FAM83H* gene **(Figure 7).**

### 3) Phenotype/Genotype correlation (isolated AI)




Typical phenotype/genotype correlation observed in patients presenting with isolated Amelogenesis imperfecta (pictures and radiographs). Patients who are carrier of *AMELX* mutations can present with different types of Amelogenesis imperfecta. Indeed, the phenotype can be either hypoplastic or hypomature depending on the mutation's localisation. When mutations occur in a *MMP20* cleavage site the AI observed is a X-linked hypomature AI. Otherwise, the phenotype observed is hypoplastic and sex dependent. In male we observed a hypoplastic AI with almost no enamel whereas female patients present a hypoplastic AI with a characteristic lyonization banding pattern (**Figure 8).**

**Figure 8 : Phenotype/Genotype correlation for isolated AI**

### 4) New proposed classification by phenotype and genetic diagnosis

We propose a new classification of Amelogenesis imperfecta based on the original Witkop phenotypical classification. Indeed, we enriched the classification with genetic data and proposed a classification based both on phenotype and genetic diagnosis **(Figure 9).**

| | | | | |
|---|---|---|---|---|
| Type I hypoplastic | IA | hypoplastic, pitted | AD | *COL17A1, COL7A1, LAMA3, LAMB3, LAMC2, LAMB2, ITGB6* |
| | IB | hypoplastic, local | AD | *ENAM* |
| | IC | hypoplastic, local | AR | *ENAM* |
| | ID | hypoplastic, smooth | AD | |
| | IE | hypoplastic, smooth | XLD | *AMELX* |
| | IF | hypoplastic, rough | AD | *AMBN* |
| | IG | enamel agenesis | AR | *FAM20A* |
| | IJ | hypoplastic | AR | *ACP4* |
| Type II hypomaturation | IIA | hypomature, pigmented | AR | *KLK4, MMP20, WDR72, ODAPH, SLC24A4, GPR68, ODAPH* |
| | IIB | hypomature | XL | *AMELX* |
| | IIC | snow-capped teeth | XL | |
| | IID | hypomature | AD | *ARHGAP6* |
| Type III hypocalcification | IIIA | hypocalcified | AD | |
| | IIIB | hypocalcified | AR | *FAM83H, AMTN* |
| Type IV hypomaturation/hypoplastic with taurodontism | IVA | hypomature/hypoplastic, taurodontism | AD | *DLX3* |
| | IVB | hypomature/hypoplastic, taurodontism | AD | |

**Figure 9 : New proposed AI classification**

## Conclusion & Discussion

NGS GenoDENT panel is a validated and cost-efficient technique offering new perspectives to understand underlying molecular mechanisms of AI. Discovering mutations in genes involved in syndromic AI (*CNNM4, WDR72, FAM20A…*) transformed patient overall care. Unravelling the genetic basis of AI shed light on Witkop's AI classification.
We reported patients with known pathogenic mutations but also patient with mutations in new candidate genes and patients presenting variants of unknown significance in known genes. For the patient with mutation in new candidate gene or with VUS developing a strategy to functionally validate the mutations will be the next step in order to improve the diagnostic rate of our tool.

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