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

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Introduction

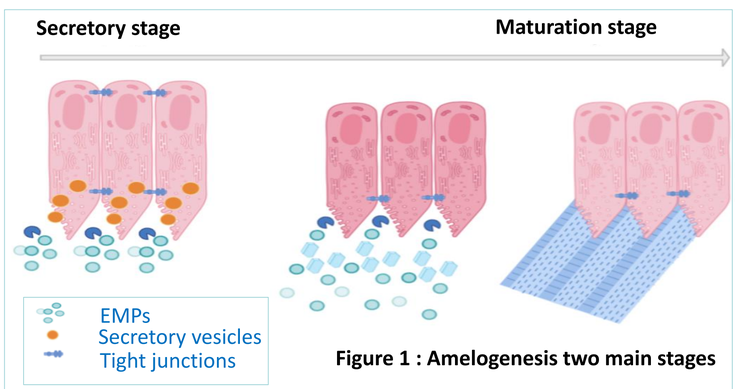


Figure 1 : Amelogenesis two main stages

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

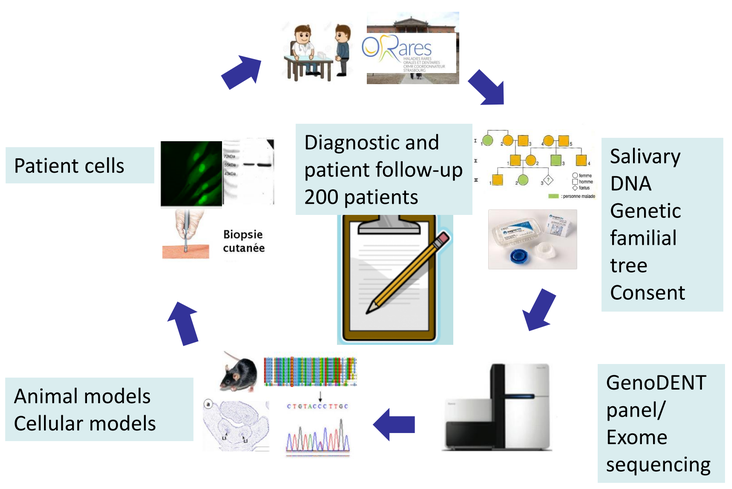


Figure 3 : Diagnostic and follow-up of patients

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

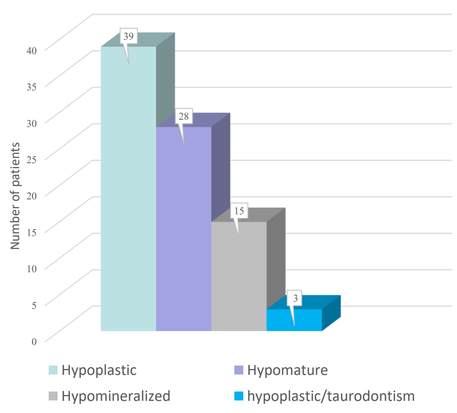
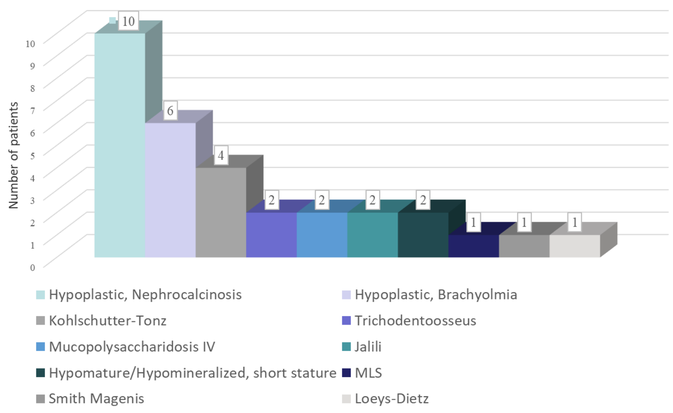


Figure 5 : Type of syndromic AI diagnosis



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

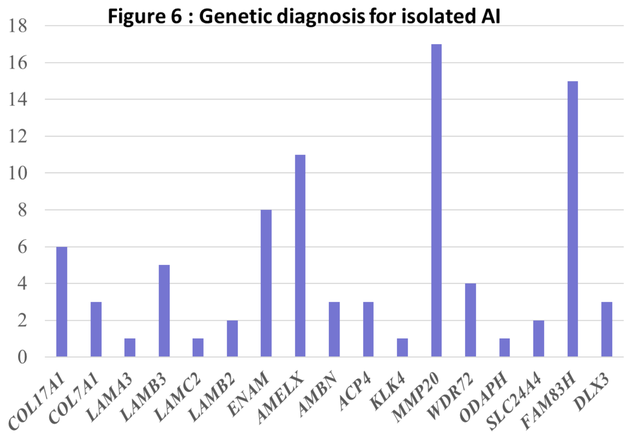
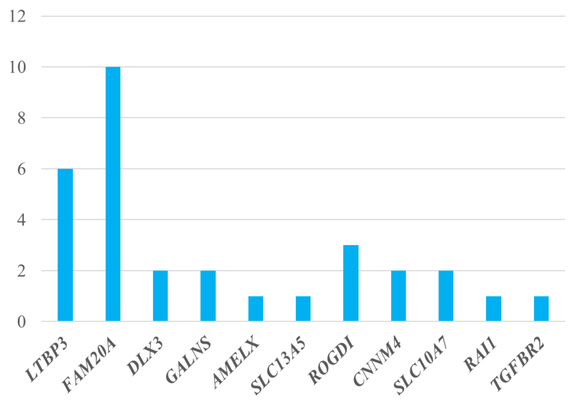
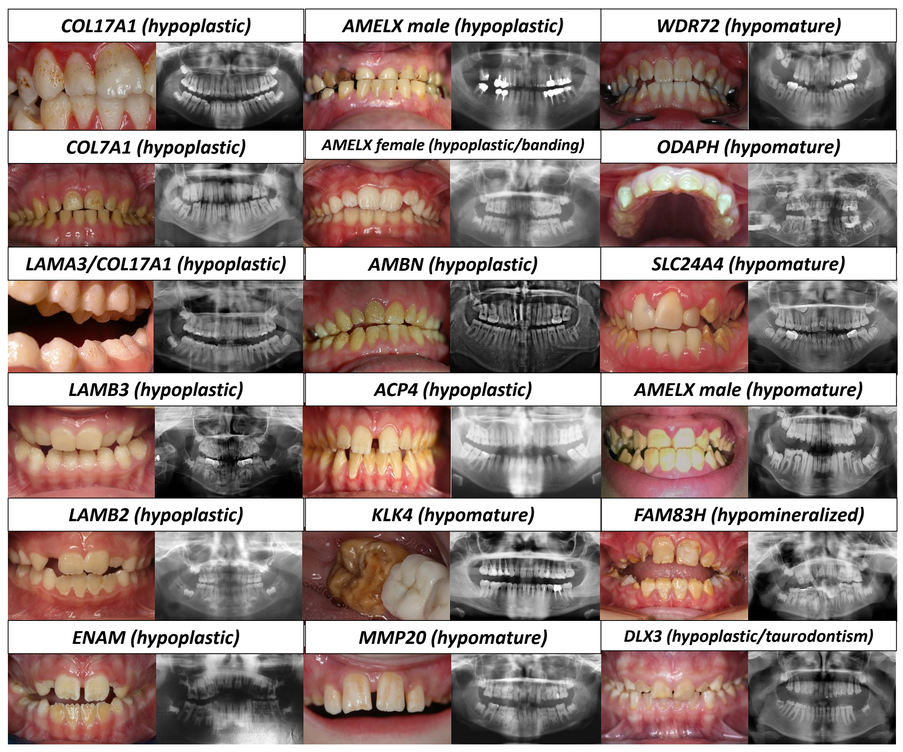


Figure 7 : Genetic diagnosis for syndromic AI



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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