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The molecular basis of non-syndromic orofacial clefts and tooth agenesis

Agnieszka Danuta Gaczkowska, Paweł Piotr Jagodziński, Adrianna Mostowska

Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poznan, Poland

ABSTRACT

Non-syndromic orofacial clefts and tooth agenesis are two of the most common craniofacial birth defects. Both of them have a complex etiology, with genetic and environmental factors involved. Additionally, the epigenetic modifications have been implicated in the pathogenesis of these structural malformations. Despite an increasing number of research studies, using a variety of methodological approaches, the role of genetic factors in the etiology of orofacial clefts and tooth agenesis is still not well elucidated. The most consistent findings across studies concerning the genetic factors influencing the risk to orofacial clefts include the association of polymorphic variants of the *IRF6* gene and the chromosomal locus 8q24.21. The major candidate gene for tooth agenesis in the European populations is *WNT10A*; its pathogenic mutations are present in more than 50% of patients with this dental anomaly. It has been found that both orofacial clefts and tooth agenesis, which co-occurrence is often reported, may share common candidate genes.

Keywords: orofacial clefts, tooth agenesis, etiology, candidate genes.

Introduction

Non-syndromic orofacial clefts (OFC) and tooth agenesis (TA) are two of the most common craniofacial birth defects [1, 2]. OFC affect 1 per 700 live births in the global population [1]. According to the *Polish Registry of Congenital Malformations, the prevalence of orofacial clefts in Poland ranges from 1/500 to 1/1000 births* [www.rejestrwad.pl]. OFC are divided into two main forms: non-syndromic cleft lip with or without cleft palate and cleft palate only [3]. The incidence of TA, excluding the lack of the third molars, varies from 1.6 to 9.6% depending on ethnic background [2]. TA can be classified based on the number of missing teeth into hypodontia (the lack of one to five teeth), oligodontia (the lack of 6 or more teeth) and anodontia (the complete absence of teeth). In this classification, the third molars are not taken into account since their absence is highly prevalent [2]. The co-occurrence of OFC and TA is often reported [4, 5]. Patients with OFC have an increased risk of dental anomalies, including alteration in tooth number, size, shape, a timing of formation and eruption comparing to the general population [6]. It has been shown that dental anomalies appear primarily in the cleft area and their prevalence is higher in left-sided OFC [7]. Additionally, in patients with OFC the agenesis of teeth *outside* the *cleft area have* also been reported to be more frequent [8]. This observation may indicate that the same molecular mechanisms may be shared in the development of the teeth, palate, and lip [8].

The etiology of non-syndromic OFC and TA is complex with genetic and environmental components [3, 9]. Additionally, the epigenetic modifications have been implicated in the pathogenesis of these structural malformations [10]. Genetic studies using a variety of research approaches, including linkage studies, candidate gene analyses, and genome-wide association studies, have identified a number of genes and chromosomal regions underlying these craniofacial anomalies [9, 11]. However, nucleotide variants of identified

candidate genes and chromosomal loci can still explain only a fraction of the predicted heritability. It has been demonstrated that both OFC and TA have a number of common candidate genes, which nucleotide variants can influence their risk [4, 5].

Across OFC' studies conducted in various populations, including the Polish population, the most consistent results were observed for nucleotide variants located in the *IRF6* gene (OMIM *607199) and the chromosomal region 8q24.21 [12–14]. The *IRF6* gene encodes a transcription factor, which is involved in the regulation of the keratinocyte proliferation-differentiation switch and formation of oral periderm [15]. It is worth noting that in a study conducted in the Latvian population, the *IRF6* variant (rs642961) located in the promoter region was found to be more frequent in individuals presenting OFC associated with tooth agenesis when compared to healthy individuals [16]. Moreover, Vieira *et al.* have demonstrated that this functional variant, disrupting an AP-2α binding site in the *IRF6* enhancer, is associated with the risk of isolated TA [12, 17, 18]. The 8q24.21 risk locus, identified by the first genome-wide association study conducted for OFC and further confirmed by a number of post-GWAS replication studies, is a gene-poor region devoid of protein-coding genes [19, 20]. Studies using mice as model organisms have demonstrated that this chromosomal locus contains very distant *cis*-acting enhancers controlling the expression of the *Myc* gene during craniofacial development [21]. Mice homozygous for the deletion including this medionasal enhancer region show mild alterations in the face morphology and *occasionally* cleft lip and palate [21]. Within the 8q24.21 chromosomal region, which nowadays is considered as a key susceptibility locus for non-syndromic OFC, the top marker associated with the risk of this anomaly is rs987525 [19]. A significant association between this intragenic variant and the co-occurrence of OFC and TA outside the cleft region was also observed [22].

The major candidate genes for non-syndromic TA include *WNT10A* (OMIM *606268), *MSX1* (OMIM *142983), *PAX9* (OMIM *167416), *AXIN2* (OMIM *604025), *EDA* (OMIM *300451) and *EDAR* (OMIM *604095). Van den Boogaard *et al.* [23] have demonstrated that the *WNT10A* mutations are present in more than 50% isolated TA cases. Pathogenic mutations within the coding region of *WNT10A* have also been identified in 62% of tooth agenesis patients from the Polish population [24]. The *WNT10A* gene is a member of the Wnt family, which consists of genes encoding secreted signaling proteins involved in a number of developmental processes during embryogenesis [25]. Interestingly, the missense mutation of the *WNT10A* gene has been associated with the increased risk of non-syndromic OFC in the Chinese population [26]. Moreover, nucleotide variants in *WNT3* (OMIM *165330), *WNT3A* (OMIM *606359), *WNT5A* (OMIM *164975), *WNT9A* (OMIM *602863), and *WNT11* (OMIM *603699) have been found to be significantly associated with non-syndromic orofacial clefts in various populations [27, 28]. Similarly, polymorphisms and mutations in the *MSX1* gene are known factors increasing the risk of non-syndromic OFC [29]. In addition, *MSX1* and two other major TA candidate genes, *PAX9* and *AXIN2*, have been associated with the co-occurrence of cleft anomalies and TA [5]. The *MSX1* and *PAX9* genes encode transcription factors that play an essential role during embryogenesis [9]. It has been demonstrated that these genes are co-expressed during craniofacial development, and the genetic interactions between their protein products are involved in the regulation of the lip formation and tooth morphogenesis [30]. *Msx1* and *Pax9* deficient mice lack all teeth, which development is arrested at the bud stage, and exhibit a number of craniofacial defects, including cleft palate [31, 32]. The *AXIN2* gene encodes a protein which is a negative regulator of the Wnt-signalling pathway [33].

Besides the genes described above, there are a number of other candidate genes and chromosomal loci underlying the co-occurrence of OFC and TA. The systemic review conducted by Phan *et al.* [5] revealed that they include among others the TGF pathway genes and the cancer predisposing gene *CDH1* (OMIM *192090).

In summary, OFC and TA are one of the most common craniofacial anomalies that share a number of common candidate genes. There is growing evidence suggesting that tooth agenesis should be considered as an extended phenotype for oral clefts [34].

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Conflict of interest statement
The authors declare no conflict of interest.

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Correspondence address:
Adrianna Mostowska, PhD
Department of Biochemistry and Molecular Biology
Poznan University of Medical Sciences
6 Swiecickiego Street, 60-781 Poznan, Poland
phone: +48 618546511
fax: +48 618546510
email: amostowska@wp.pl