

EDITORIAL

Craniofacial genetics: Where have we been and where are we going?

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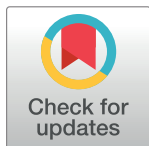
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Looking at faces is always illuminating. Perhaps, this is because our faces reveal so much about us, ranging from our evolutionary history to our embryological development, genetic endowment, propensity for disease, current health status, and exposures over our lifespan. The structure of our faces may even reveal insights into our personalities—an idea that stretches back to the ancient Greeks. The face is a complex constellation of parts serving functions as diverse as sight, hearing, smell, breathing, nourishment and digestion, protection, and communication. Despite our collective fascination, we still have limited understanding of the molecular machinery that controls how our faces form or how morphological variation in facial features arises, from the typical and often subtle differences that endow each of us with our unique facial appearance to the rare craniofacial malformations seen in the clinic. However, we are making incredible progress in these areas, and the pace of discovery is poised to accelerate rapidly, facilitated by the emergence of high-throughput experimental methods, advances in computational modeling, and the investment and availability of large-scale craniofacial data resources, (e.g., the FaceBase Consortium).

In recognition of these advances, *PLOS Genetics* invited us to put together a special collection on the theme of craniofacial genetics. This collection was not meant to be exhaustive but rather a curated selection of published papers covered in PLOS journals deemed (by us) to be particularly germane. Thus, the selection is, by its nature, highly subjective, reflecting our particular areas of interest: craniofacial morphogenesis, dysmorphology and syndromes, and normal human facial variation.

The genes and pathways controlling craniofacial morphogenesis: Zebrafish as a model

The zebrafish has emerged as a potent addition to the traditional mouse model for studies of craniofacial morphogenesis. The main features include external development and optical transparency, which both facilitate imaging of live embryos and amenability to forward and reverse genetic methods. Also, the zebrafish larval face is simpler than that of a newborn human at the time feeding begins [1]. Many of the mechanisms governing early growth and patterning of the face are shared across all vertebrates. Although there are unique structures in zebrafish, mice, and humans, fundamental cellular processes are conserved. For instance, convergence of frontonasal and maxillary neural crest occurs analogously in the formation of the mammalian hard palate and the zebrafish ethmoid plate. The soft palate, separating the nasal cavity and the oral cavity in mammals, is absent in zebrafish. Nonetheless, zebrafish may still



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be useful in studying genetic underpinnings of cleft (soft) palate, as the gene regulatory networks governing differentiation of relevant tissues are similar between zebrafish and mammals [2]. Exemplifying this point, the gene regulatory network governing differentiation of the mammalian superficial oral epithelium (oral periderm), where a large subset of genes associated with risk for cleft palate are expressed, has many shared features with that of zebrafish periderm [3–6]. This means that many insights into zebrafish development, gleaned from its impressive experimental toolkit, will be relevant to the development of the mammalian face and, in some cases, to the genetic underpinnings of orofacial clefting.

The external development and transparency of zebrafish embryos permits exquisite imaging by high-resolution confocal microscopy. One application is fate mapping using photo-convertible dyes [7]. Reporters built from the regulatory elements of appropriate genes have been used extensively to mark all neural crest cells [8,9], and, more recently, subsets of them that are differentiated as chondrocytes [10] or bone [11]. Use of such tools, and vital dyes, in normal animals has enabled the study of changes in cell organization that occur as a stream of cranial neural crest converts into a skeletal element of precise size and shape [11]. Similar analyses in mutants permit identification of the exact steps that go awry, facilitating identification of the underlying cellular defects [12]. Another potent application of imaging is the development of reporter lines that reveal a response to particular extracellular signals relevant to craniofacial patterning, including BMPs [13], FGFs [14], WNTs [15], and Hedgehog [16]. These tools permit investigations that would be much harder to carry out in mice.

Another experimental strength of zebrafish is that they are well suited to forward genetic screens, a potent method to identify genes involved in craniofacial development, including of the face. Even in an era where targeted mutagenesis is becoming increasingly efficient, random mutagenesis screens make an important contribution because they can yield alleles that would never be generated intentionally. Hypomorphic alleles can reveal a function in craniofacial morphogenesis for a gene product in which a total loss of function allele leads to death prior to craniofacial development. Morphology-based screens in the late 1990s revealed more than 80 mutants (as complementation groups) with grossly defective head shape [17–20]. Molecular characterization of mutants from these screens and subsequent ones that evaluated subtler phenotypes based on gene expression and histology revealed a large number of genes previously shown to be essential for development of the mammalian face, showing that these pathways are conserved. Excitingly, such efforts have also identified several genes not previously implicated in craniofacial development. Such genes include histone variant H3.3 [21], Rabconnectin-3a [22], and Hdac1 [23]. These genes are likely to be important for mammalian facial development as well. There is no reason to think such screens have been saturated, and additional, more elaborate reporter-based screens may reveal genes missed in earlier screens.

Zebrafish are also useful for loss-of-function studies; until recently, this was most frequently achieved by antisense morpholinos but increasingly by targeted mutagenesis mediated by clustered regularly interspersed short palindromic repeats (CRISPR)/Cas9 [24]. There is debate in the field regarding why the phenotypes induced by morpholinos and those induced by targeted mutagenesis are frequently not congruent, with the latter often less severe. One possibility is that the morphant phenotype is an artifact from an off target effect [25]; another is that targeted mutagenesis, but not morpholinos, induces a compensatory mechanism [26, 27]. Currently, a conservative approach is to use morpholinos only when they phenocopy a mutant [28]. More than 30 genes associated with craniofacial disorders also result in craniofacial dysmorphogenesis in zebrafish [1,29]. In many cases, confirmation of the morpholino oligonucleotide-induced phenotype in a targeted mutant remains to be achieved. Once it has been, the exquisite imaging methods available in zebrafish should permit relatively facile discovery of the function of each of these genes in craniofacial development.

The genetics of craniofacial malformations and syndromes

Over the last 15 years, we have witnessed a dramatic increase in gene discovery for all manners of craniofacial phenotypes due to the development and application of high-throughput genomic technologies. Beginning in 2005, genome-wide association studies (GWAS) have produced strong evidence that common DNA sequence variants influence risk of common traits and diseases. The first application of GWAS to craniofacial phenotypes came in 2009 with back-to-back publications on nonsyndromic orofacial clefts (OFCs) [30,31]. Additional GWAS have been published at a consistent rate, collectively identifying over 25 risk loci across diverse population groups [32–43]. The more recent application of next-generation sequencing (NGS) technology welcomed rapid gene discovery for rare diseases, including those with extreme rarity, clinical heterogeneity, and those without strong family history that were not amenable to linkage. The major contribution of GWAS and discovery of etiological mutations by NGS has been the identification of novel candidate genes and new hypotheses about the mechanisms contributing to craniofacial development; these discoveries complement the insights gained from forward genetic screens in zebrafish mentioned above.

Novel findings for both common and rare craniofacial disorders have provided new etiological hypotheses and new insights into craniofacial phenotypes but also present new challenges for research. First, the discovery of new candidate genes has outpaced the functional studies required to understand the cellular and molecular mechanisms for both common and rare diseases. This is especially true for etiological variants occurring in noncoding regulatory parts of the genome, which we know considerably less about and for which we lack robust bioinformatic tools for annotation. Second, both GWAS and NGS have revealed a complexity to phenotypic presentations. For GWAS, identified loci may act broadly on the craniofacial complex, while others have very precise effects. In the example of OFCs, the *FOXE1* locus on 9q22 increases risk for all types of OFCs (including cleft palate only) [34, 44], whereas the *GREM1* locus on 15q13 appears to have specificity for cleft lip and palate [45]. For rare diseases, NGS has clarified unusual presentations of syndromes. In some cases, sequencing reveals multiple, independent molecular diagnoses in individuals previously thought to have a novel or new variation of a single syndrome. As a result, we foresee a growing list of genes in which genetic variants contribute to multiple diseases, including syndromic and nonsyndromic forms of craniofacial disorders, leading to new questions as to the mechanisms by which certain genotypes lead to observed phenotypes. Addressing these questions will require continued detailed phenotyping, large-scale genotyping and sequencing efforts, the development of new methods to identify genetic modifiers or interactions, and high-throughput functional assays in model systems.

The complex genetics underlying normal-range human craniofacial morphology

Subtle variations characterize virtually every aspect of human facial morphology, the combinatorial possibilities resulting in a seemingly endless diversity of facial forms. The suggestion has been made that human faces are indeed more variable than the faces of other species and compared to other parts of our body [46]—a fact with potential evolutionary implications. It is therefore remarkable that so little is known about the biological factors underlying “normal-range” facial variation. There is no doubt that our genes play a major role; plain evidence for this can be found in the strong facial resemblance among members of our own families. More formally, we know from twin studies which aspects of our facial morphology are most and least heritable [47]. The genetic basis of human facial malformations and syndromes (discussed above) provides yet another line of evidence indicating the critical role of genes in shaping the human face.

The effort to identify the genes that influence normal-range facial phenotypes is important for several reasons. First, our understanding of how genes orchestrate facial morphogenesis is incomplete (discussed above). Gene mapping studies can provide developmental and cell biologists with new candidates for investigation (which genes to focus on). Moreover, such studies can reveal which aspects of facial morphology are likely to be impacted by specific variants and therefore the cell populations and tissues in which to look for effects. Second, an improved understanding of the genetic basis of normal-range variation can help elucidate the etiology of craniofacial malformations. Many craniofacial disorders are characterized by highly variable phenotypic expression. In Treacher Collins syndrome, for example, the same *TCOF1* mutation can impact the face with dramatically different levels of severity [48]. Such phenotypic variability may be due to the impact of many other normally functioning genes that influence how the face grows acting either independently or interactively with the mutated gene (and environmental factors) to produce a final outcome. Third, in the future, it may be possible to use information gleaned from facial gene mapping studies to create predictive models of facial features. We are not able to do this yet, but one can imagine the potential applications: recreating faces from DNA harvested from the bones of ancestors or left at a crime scene or residing in a genomic data repository like dbGaP, having the ability to predict an unborn child's face or the face of a distant relative, or incorporating a patient's genomic data into treatment planning at the orthodontist. Exploring such ideas is exciting but in many cases will be associated with important ethical and privacy implications.

The first genetic studies of normal-range human facial features were focused on candidate genes implicated in craniofacial syndromes or from transgenic mouse models with severe facial phenotypes [49–52]. Several of these early studies were featured in PLOS journals. The eventual emergence of large data sets containing both facial images—which allow facial morphology to be quantified in various ways—and dense genomic markers accelerated the pace of discovery by allowing for genome-scale analyses [53]. In 2012, the first GWAS of normal-range human facial shape were published [54–55]; one of these seminal studies by Liu and colleagues [55] appeared in *PLOS Genetics*. Of the handful of genome-wide significant loci identified by these 2 studies, the signal at *PAX3* was replicated. Common genetic variants at *PAX3* were associated with the shape of the nasal root—the uppermost part of the nose located between the eyes. This is noteworthy because mutations in *PAX3* can result in Waardenburg Syndrome Type 1, where one of the cardinal features is dysmorphology of the nasal root area. Several subsequent GWAS have now been conducted [56–59]. Each of these studies has identified a handful of loci, many of which contained at least one strong craniofacial candidate gene, associated with various measured facial traits. A recent study [60] using a novel data-driven approach to measuring 3D facial images based on machine learning principles identified 38 genome-wide significant loci, 15 of which were independently replicated. A number of the 15 replicated loci contained genes that have been implicated in early craniofacial development and in human craniofacial syndromes (e.g., *TBX15*, *SOX9*, *PAX3*, *DLX6*). An important finding from this study was that the effects of genetic variants on facial morphology tend to be highly specific, even within a single structural component like the nose. Another important finding was that variants at these 15 loci tended to show preferential activity in human cranial neural crest cells—a critical cell population for building the face.

As with most common traits [61], the implicated variants from the above facial GWAS tend to reside in noncoding (regulatory) regions of the genome. For example, many variants at the 15 aforementioned facial loci were in or near enhancers known to play a role in human craniofacial development [60]. This makes sense because variants that regulate the expression of intact genes—without altering the gene's protein structure—would be expected to result in the kind of subtle modifications that characterize normal-range facial variation. This fact raises a

number of challenges for identifying the likely functional variants, since much of the noncoding genome is still poorly characterized. Moreover, at any given locus, there may be many related variants that show evidence of association simply due to their proximity to the true functional variant(s). We expect this problem to only be exacerbated with the analysis of larger cohorts, which will likely reveal many more loci. The application of newer high-throughput functional approaches designed to probe the noncoding portions of the genome (CRISPR Cas9-based tools; STARR-seq) will be needed here to help sort through and make sense of these GWAS results [62].

Major breakthroughs are taking place in the area of craniofacial genetics, and we expect a rapid acceleration of discovery over the next few years. PLOS journals have played a key role in advancing the science, not only by providing a venue to publish cutting edge craniofacial research—some of which is highlighted in this collection—but also by making the availability of underlying data a key aspect and expectation of the publication process. The importance of open access and data sharing is critical for the transparency and reproducibility of our work and our field. These values help ensure that our scientific findings will eventually find their way to improving people's lives.

References

1. Mork L, Crump G. Zebrafish craniofacial development: a window into early patterning. *Curr Top Dev Biol.* 2015; 115:235–269. <https://doi.org/10.1016/bs.ctdb.2015.07.001> PMID: 26589928
2. Swartz ME, Sheehan-Rooney K, Dixon MJ, Eberhart JK. Examination of a palatogenic gene program in zebrafish. *Dev Dyn.* 2011; 240:2204–2220. <https://doi.org/10.1002/dvdy.22713> PMID: 22016187
3. de la Garza G, Schleiffarth JR, Dunnwald M, Mankad A, Weirather JL, Bonde G, et al. Interferon regulatory factor 6 promotes differentiation of the periderm by activating expression of Grainyhead-like 3. *J Invest Derm.* 2013; 133:68–77. <https://doi.org/10.1038/jid.2012.269> PMID: 22931925
4. Peyrard-Janvid M, Leslie EJ, Kousa YA, Smith TL, Dunnwald M, Magnusson M, et al. Dominant mutations in GRHL3 cause Van der Woude Syndrome and disrupt oral periderm development. *Am J Hum Genet.* 2014; 94:23–32. <https://doi.org/10.1016/j.ajhg.2013.11.009> PMID: 24360809
5. Richardson RJ, Dixon J, Jiang R, Dixon MJ. Integration of IRF6 and Jagged2 signalling is essential for controlling palatal adhesion and fusion competence. *Hum Mol Genet.* 2009; 18:2632–2642. <https://doi.org/10.1093/hmg/ddp201> PMID: 19439425
6. Sabel JL, d'Alencon C, O'Brien EK, Otterloo EV, Lutz K, Cuykendall TN, et al. Maternal Interferon Regulatory Factor 6 is required for the differentiation of primary superficial epithelia in Danio and Xenopus embryos. *Dev Biol.* 2009; 325:249–262. <https://doi.org/10.1016/j.ydbio.2008.10.031> PMID: 19013452
7. Dougherty M, Kamel G, Shubinets V, Hickey G, Grimaldi M, Liao EC. Embryonic fate map of first pharyngeal arch structures in the sox10: kaede zebrafish transgenic model. *J Craniofac Surg.* 2012; 23:1333–1337. <https://doi.org/10.1097/SCS.0b013e318260f20b> PMID: 22948622
8. Wada N, Javidan Y, Nelson S, Carney TJ, Kelsh RN, Schilling TF. Hedgehog signaling is required for cranial neural crest morphogenesis and chondrogenesis at the midline in the zebrafish skull. *Development.* 2005; 132:3977–3988. <https://doi.org/10.1242/dev.01943> PMID: 16049113
9. Lawson ND, Weinstein BM. In vivo imaging of embryonic vascular development using transgenic zebrafish. *Dev Biol.* 2002; 248:307–318. PMID: 12167406
10. Eames BF, DeLaurier A, Ullmann B, Huycke TR, Nichols JT, Dowd J, et al. FishFace: interactive atlas of zebrafish craniofacial development at cellular resolution. *BMC Dev Biol.* 2013; 13:23. <https://doi.org/10.1186/1471-213X-13-23> PMID: 23714426
11. Kimmel CB, DeLaurier A, Ullmann B, Dowd J, McFadden M. Modes of developmental outgrowth and shaping of a craniofacial bone in zebrafish. *PLoS ONE.* 2010; 5:e9475. <https://doi.org/10.1371/journal.pone.0009475> PMID: 20221441
12. Le Pabic P, Ng C, Schilling TF. Fat-Dachsous signaling coordinates cartilage differentiation and polarity during craniofacial development. *PLoS Genet.* 2014; 10:e1004726. <https://doi.org/10.1371/journal.pgen.1004726> PMID: 25340762
13. Alexander C, Zuniga E, Blitz IL, Wada N, Le Pabic P, Javidan Y, et al. Combinatorial roles for BMPs and Endothelin 1 in patterning the dorsal-ventral axis of the craniofacial skeleton. *Development.* 2011; 138:5135–5146. <https://doi.org/10.1242/dev.067801> PMID: 22031543

14. Molina GA, Watkins SC, Tsang M. Generation of FGF reporter transgenic zebrafish and their utility in chemical screens. *BMC Dev Biol.* 2007; 7:62. <https://doi.org/10.1186/1471-213X-7-62> PMID: [17553162](https://pubmed.ncbi.nlm.nih.gov/17553162/)
15. Moro E, Ozhan-Kizil G, Mongera A, Beis D, Wierzbicki C, Young RM, et al. In vivo Wnt signaling tracing through a transgenic biosensor fish reveals novel activity domains. *Dev Biol.* 2012; 366:327–340. <https://doi.org/10.1016/j.ydbio.2012.03.023> PMID: [22546689](https://pubmed.ncbi.nlm.nih.gov/22546689/)
16. Schwend T, Loucks EJ, Ahlgren SC. Visualization of Gli activity in craniofacial tissues of hedgehog-pathway reporter transgenic zebrafish. *PLoS ONE.* 2010; 5:e14396. <https://doi.org/10.1371/journal.pone.0014396> PMID: [21203590](https://pubmed.ncbi.nlm.nih.gov/21203590/)
17. Neuhauss SC, Solnica-Krezel L, Schier AF, Zwartkruis F, Stemple DL, Malicki J, et al. Mutations affecting craniofacial development in zebrafish. *Development.* 1996; 123:357–367. PMID: [9007255](https://pubmed.ncbi.nlm.nih.gov/9007255/)
18. Piotrowski T, Schilling TF, Brand M, Jiang YJ, Heisenberg CP, Beuchle D, et al. Jaw and branchial arch mutants in zebrafish II: anterior arches and cartilage differentiation. *Development.* 1996; 123:345–356. PMID: [9007254](https://pubmed.ncbi.nlm.nih.gov/9007254/)
19. Driever W, Solnica-Krezel L, Schier AF, Neuhauss SC, Malicki J, Stemple DL, et al. A genetic screen for mutations affecting embryogenesis in zebrafish. *Development.* 1996; 123:37–46. PMID: [9007227](https://pubmed.ncbi.nlm.nih.gov/9007227/)
20. Schilling TF, Piotrowski T, Grandel H, Brand M, Heisenberg CP, Jiang YJ, et al. Jaw and branchial arch mutants in zebrafish I: branchial arches. *Development.* 1996; 123:329–344. PMID: [9007253](https://pubmed.ncbi.nlm.nih.gov/9007253/)
21. Cox SG, Kim H, Garnett AT, Medeiros DM, An W, Crump JG. An essential role of variant histone H3.3 for ectomesenchyme potential of the cranial neural crest. *PLoS Genet.* 2012; 8:e1002938. <https://doi.org/10.1371/journal.pgen.1002938> PMID: [23028350](https://pubmed.ncbi.nlm.nih.gov/23028350/)
22. Tuttle AM, Hoffman TL, Schilling TF. Rabconnectin-3a regulates vesicle endocytosis and canonical Wnt signaling in zebrafish neural crest migration. *PLoS Biol.* 2014; 12:e1001852. <https://doi.org/10.1371/journal.pbio.1001852> PMID: [24802872](https://pubmed.ncbi.nlm.nih.gov/24802872/)
23. Ignatius MS, Unal Eroglu A, Malireddy S, Gallagher G, Nambiar RM, Henion PD. Distinct functional and temporal requirements for zebrafish Hdac1 during neural crest-derived craniofacial and peripheral neuron development. *PLoS ONE.* 2013; 8:e63218. <https://doi.org/10.1371/journal.pone.0063218> PMID: [23667588](https://pubmed.ncbi.nlm.nih.gov/23667588/)
24. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, et al. Multiplex genome engineering using CRISPR/Cas systems. *Science.* 2013; 339:819–823. <https://doi.org/10.1126/science.1231143> PMID: [23287718](https://pubmed.ncbi.nlm.nih.gov/23287718/)
25. Kok FO, Shin M, Ni CW, Gupta A, Grosse AS, van Impel A, et al. Reverse genetic screening reveals poor correlation between morpholino-induced and mutant phenotypes in zebrafish. *Dev Cell.* 2015; 32:97–108. <https://doi.org/10.1016/j.devcel.2014.11.018> PMID: [25533206](https://pubmed.ncbi.nlm.nih.gov/25533206/)
26. El-Brolosy MA, Stainier DYR. Genetic compensation: A phenomenon in search of mechanisms. *PLoS Genet.* 2017; 13:e1006780. <https://doi.org/10.1371/journal.pgen.1006780> PMID: [28704371](https://pubmed.ncbi.nlm.nih.gov/28704371/)
27. Rossi A, Kontarakis Z, Gerri C, Nolte H, Holper S, Kruger M, et al. Genetic compensation induced by deleterious mutations but not gene knockdowns. *Nature.* 2015; 524:230–233. <https://doi.org/10.1038/nature14580> PMID: [26168398](https://pubmed.ncbi.nlm.nih.gov/26168398/)
28. Stainier DYR, Raz E, Lawson ND, Ekker SC, Burdine RD, Eisen JS, et al. Guidelines for morpholino use in zebrafish. *PLoS Genet.* 2017; 13:e1007000. <https://doi.org/10.1371/journal.pgen.1007000> PMID: [29049395](https://pubmed.ncbi.nlm.nih.gov/29049395/)
29. Duncan KM, Mukherjee K, Cornell RA, Liao EC. Zebrafish models of orofacial clefts. *Dev Dyn.* 2017; 246:897–914. <https://doi.org/10.1002/dvdy.24566> PMID: [28795449](https://pubmed.ncbi.nlm.nih.gov/28795449/)
30. Grant SF, Wang K, Zhang H, Glaberson W, Annaiah K, Kim CE, et al. A genome-wide association study identifies a locus for nonsyndromic cleft lip with or without cleft palate on 8q24. *J Pediatr.* 2009; 155:909–913. <https://doi.org/10.1016/j.jpeds.2009.06.020> PMID: [19656524](https://pubmed.ncbi.nlm.nih.gov/19656524/)
31. Birnbaum S, Ludwig KU, Reutter H, Herms S, Steffens M, Rubini M, et al. Key susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24. *Nat Genet.* 2009; 41:473–477. <https://doi.org/10.1038/ng.333> PMID: [19270707](https://pubmed.ncbi.nlm.nih.gov/19270707/)
32. Yu Y, Zuo X, He M, Gao J, Fu Y, Qin C, et al. Genome-wide analyses of non-syndromic cleft lip with palate identify 14 novel loci and genetic heterogeneity. *Nat Commun.* 2017; 8:14364. <https://doi.org/10.1038/ncomms14364> PMID: [28232668](https://pubmed.ncbi.nlm.nih.gov/28232668/)
33. Ludwig KU, Böhmer AC, Bowes J, Nikolic M, Ishorst N, Wyatt N, et al. Imputation of orofacial clefting data identifies novel risk loci and sheds light on the genetic background of cleft lip ± cleft palate and cleft palate only. *Hum Mol Genet.* 2017; 26:829–842. <https://doi.org/10.1093/hmg/ddx012> PMID: [28087736](https://pubmed.ncbi.nlm.nih.gov/28087736/)
34. Leslie EJ, Carlson JC, Shaffer JR, Butali A, Buxó CJ, Castilla EE, et al. Genome-wide meta-analyses of nonsyndromic orofacial clefts identify novel associations between FOXE1 and all orofacial clefts, and TP63 and cleft lip with or without cleft palate. *Hum Genet.* 2017; 136:275–286. <https://doi.org/10.1007/s00439-016-1754-7> PMID: [28054174](https://pubmed.ncbi.nlm.nih.gov/28054174/)

35. Leslie EJ, Liu H, Carlson JC, Shaffer JR, Feingold E, Wehby G, et al. A Genome-wide Association Study of Nonsyndromic Cleft Palate Identifies an Etiologic Missense Variant in GRHL3. *Am J Hum Genet.* 2016; 98:744–754. <https://doi.org/10.1016/j.ajhg.2016.02.014> PMID: 27018472
36. Leslie EJ, Carlson JC, Shaffer JR, Feingold E, Wehby G, Laurie CA, et al. A multi-ethnic genome-wide association study identifies novel loci for non-syndromic cleft lip with or without cleft palate on 2p24.2, 17q23 and 19q13. *Hum Mol Genet.* 2016; 25:2862–2872. <https://doi.org/10.1093/hmg/ddw104> PMID: 27033726
37. Wolf ZT, Brand HA, Shaffer JR, Leslie EJ, Arzi B, Willet CE, et al. Genome-wide association studies in dogs and humans identify ADAMTS20 as a risk variant for cleft lip and palate. *PLoS Genet.* 2015; 11: e1005059. <https://doi.org/10.1371/journal.pgen.1005059> PMID: 25798845
38. Sun Y, Huang Y, Yin A, Pan Y, Wang Y, Wang C, et al. Genome-wide association study identifies a new susceptibility locus for cleft lip with or without a cleft palate. *Nat Commun.* 2015; 6:6414. <https://doi.org/10.1038/ncomms7414> PMID: 25775280
39. Ludwig KU, Mangold E, Herms S, Nowak S, Reutter H, Paul A, et al. Genome-wide meta-analyses of nonsyndromic cleft lip with or without cleft palate identify six new risk loci. *Nat Genet.* 2012; 44:968–971. <https://doi.org/10.1038/ng.2360> PMID: 22863734
40. Camargo M, Rivera D, Moreno L, Lidral AC, Harper U, Jones M, et al. GWAS reveals new recessive loci associated with non-syndromic facial clefting. *Eur J Med Genet.* 2012; 55:510–514. <https://doi.org/10.1016/j.ejmg.2012.06.005> PMID: 22750566
41. Beaty TH, Ruczinski I, Murray JC, Marazita ML, Munger RG, Hetmanski JB, et al. Evidence for gene-environment interaction in a genome wide study of nonsyndromic cleft palate. *Genet Epidemiol.* 2011; 35:469–78. <https://doi.org/10.1002/gepi.20595> PMID: 21618603
42. Mangold E, Ludwig KU, Birnbaum S, Baluardo C, Ferrian M, Herms S, et al. Genome-wide association study identifies two susceptibility loci for nonsyndromic cleft lip with or without cleft palate. *Nat Genet.* 2010; 42:24–26. <https://doi.org/10.1038/ng.506> PMID: 20023658
43. Beaty TH, Murray JC, Marazita ML, Munger RG, Ruczinski I, Hetmanski JB, et al. A genome-wide association study of cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. *Nat Genet.* 2010; 42:525–529. <https://doi.org/10.1038/ng.580> PMID: 20436469
44. Moreno LM, Mansilla MA, Bullard SA, Cooper ME, Busch TD, Machida J, et al. FOXE1 association with both isolated cleft lip with or without cleft palate, and isolated cleft palate. *Hum Mol Genet.* 2009; 18:4879–4896. <https://doi.org/10.1093/hmg/ddp444> PMID: 19779022
45. Ludwig KU, Ahmed ST, Böhmer AC, Sangani NB, Varghese S, Klamt J, et al. Meta-analysis Reveals Genome-Wide Significance at 15q13 for Nonsyndromic Clefting of Both the Lip and the Palate, and Functional Analyses Implicate GREM1 As a Plausible Causative Gene. *PLoS Genet.* 2016; 12: e1005914. <https://doi.org/10.1371/journal.pgen.1005914> PMID: 26968009
46. Sheehan MJ, Nachman MW. Morphological and population genomic evidence that human faces have evolved to signal individual identity. *Nat Commun.* 2014; 5:4800. <https://doi.org/10.1038/ncomms5800> PMID: 25226282
47. Tsagkrasoulis D, Hysi P, Spector T, Montana G. Heritability maps of human face morphology through large-scale automated three-dimensional phenotyping. *Sci Rep.* 2017; 7:45885. <https://doi.org/10.1038/srep45885> PMID: 28422179
48. Schlump JU, Stein A, Hehr U, Karen T, Möller-Hartmann C, Elcioglu NH, et al. Treacher Collins syndrome: clinical implications for the paediatrician—a new mutation in a severely affected newborn and comparison with three further patients with the same mutation, and review of the literature. *Eur J Pediatr.* 2012; 171:1611–1618. <https://doi.org/10.1007/s00431-012-1776-7> PMID: 22729243
49. Coussens AK, van Daal A. Linkage disequilibrium analysis identifies an FGFR1 haplotype-tag SNP associated with normal variation in craniofacial shape. *Genomics* 2005; 85:563–573. <https://doi.org/10.1016/j.ygeno.2005.02.002> PMID: 15820308
50. Kang EH, Yamaguchi T, Tajima A, Nakajima T, Tomoyasu Y, Watanabe M, et al. Association of the growth hormone receptor gene polymorphisms with mandibular height in a Korean population. *Arch Oral Biol.* 2009; 54:556–562. <https://doi.org/10.1016/j.archoralbio.2009.03.002> PMID: 19344888
51. Peng S, Tan J, Hu S, Zhou H, Guo J, Jin L, et al. Detecting genetic association of common human facial morphological variation using high density 3D image registration. *PLoS Comput Biol.* 2013; 9: e1003375. <https://doi.org/10.1371/journal.pcbi.1003375> PMID: 24339768
52. Claes P, Liberton DK, Daniels K, Rosana KM, Quillen EE, Pearson LN, et al. Modeling 3D facial shape from DNA. *PLoS Genet.* 2014; 10:e1004224. <https://doi.org/10.1371/journal.pgen.1004224> PMID: 24651127
53. Hochheiser H, Aronow BJ, Artinger K, Beaty TH, Brinkley JF, Chai Y, et al. The FaceBase Consortium: A comprehensive program to facilitate craniofacial research. *Dev Biol.* 2011; 355:175–182. <https://doi.org/10.1016/j.ydbio.2011.02.033> PMID: 21458441

54. Paternoster L, Zhurov AI, Toma AM, Kemp JP, St Pourcain B, Timpson NJ, et al. Genome-wide association study of three-dimensional facial morphology identifies a variant in PAX3 associated with nasion position. *Am J Hum Genet.* 2012; 90:478–485. <https://doi.org/10.1016/j.ajhg.2011.12.021> PMID: [22341974](https://pubmed.ncbi.nlm.nih.gov/22341974/)
55. Liu F, van der Lijn F, Schurmann C, Zhu G, Chakravarty MM, Hysi PG, et al. A genome-wide association study identifies five loci influencing facial morphology in Europeans. *PLoS Genet.* 2012; 8:e1002932. <https://doi.org/10.1371/journal.pgen.1002932> PMID: [23028347](https://pubmed.ncbi.nlm.nih.gov/23028347/)
56. Adhikari K, Fuentes-Guajardo M, Quinto-Sánchez M, Mendoza-Revilla J, Camilo Chacón-Duque J, Acuña-Alonzo V, et al. A genome-wide association scan implicates DCHS2, RUNX2, GLI3, PAX1 and EDAR in human facial variation. *Nat Commun.* 2016; 7:11616. <https://doi.org/10.1038/ncomms11616> PMID: [27193062](https://pubmed.ncbi.nlm.nih.gov/27193062/)
57. Shaffer JR, Orlova E, Lee MK, Leslie EJ, Raffensperger ZD, Heike CL, et al. Genome-wide association study reveals multiple loci influencing normal human facial morphology. *PLoS Genet.* 2016; 12:e1006149. <https://doi.org/10.1371/journal.pgen.1006149> PMID: [27560520](https://pubmed.ncbi.nlm.nih.gov/27560520/)
58. Cole JB, Manyama M, Kimwaga E, Mathayo J, Larson JR, Liberton DK, et al. Genomewide association study of African children Identifies association of SCHIP1 and PDE8A with facial size and shape. *PLoS Genet.* 2016; 12:e1006174. <https://doi.org/10.1371/journal.pgen.1006174> PMID: [27560698](https://pubmed.ncbi.nlm.nih.gov/27560698/)
59. Lee MK, Shaffer JR, Leslie EJ, Orlova E, Carlson JC, Feingold E, et al. Genome-wide association study of facial morphology reveals novel associations with FREM1 and PARK2. *PLoS ONE.* 2017; 12:e0176566. <https://doi.org/10.1371/journal.pone.0176566> PMID: [28441456](https://pubmed.ncbi.nlm.nih.gov/28441456/)
60. Claes P, Roosenboom J, White JD, Swigut T, Sero D, Li J, et al. Genome-wide mapping of global-to-local genetic effects on human facial shape. *Nat Genet.* 2018; 50:414–423. <https://doi.org/10.1038/s41588-018-0057-4> PMID: [29459680](https://pubmed.ncbi.nlm.nih.gov/29459680/)
61. Gusev A, Lee SH, Trynka G, Finucane H, Vilhjálmsón BJ, Xu H, et al. Partitioning heritability of regulatory and cell-type-specific variants across 11 common diseases. *Am J Hum Genet.* 2014; 95:535–552. <https://doi.org/10.1016/j.ajhg.2014.10.004> PMID: [25439723](https://pubmed.ncbi.nlm.nih.gov/25439723/)
62. Ipe J, Swart M, Burgess KS, Skaar TC. High-Throughput Assays to Assess the Functional Impact of Genetic Variants: A Road Towards Genomic-Driven Medicine. *Clin Transl Sci.* 2017; 10:67–77. <https://doi.org/10.1111/cts.12440> PMID: [28213901](https://pubmed.ncbi.nlm.nih.gov/28213901/)