Molecular biology to infer phenotypes of forensic and ancient remains in bioarchaeology – Review

Gabriel Dorado [1], Fernando Luque [2], Plácido Pascual [3], Inmaculada Jiménez [4], Francisco Javier S. Sánchez-Cañete [5], Patricia Raya [6], Jesús Sáiz [7], Adela Sánchez [7], Teresa E. Rosales [8], Víctor F. Vásquez [9], Pilar Hernández [10]

[1] Author for correspondence, Dep. Bioquímica y Biología Molecular, Campus Rabanales C6-1-E17, Campus de Excelencia Internacional Agroalimentario (ceiA3), Universidad de Córdoba, 14071 Córdoba (Spain), eMail: <bb1dopeg@uco.es>; [2] Laboratorio de Producción y Sanidad Animal de Córdoba, Ctra. Madrid-Cádiz km 395, 14071 Córdoba; [3] Laboratorio Agroalimentario de Córdoba, Consejería de Agricultura y Pesca, Junta de Andalucía, 14004 Córdoba; [4] IES Puertas del Campo, Avda. San Juan de Dios 1, 51001 Ceuta; [5] EE.PP. Sagrada Familia de Baena, Avda. Padre Villoslada 22, 14850 Baena (Córdoba); [6] Dep. Radiología y Medicina Física, Unidad de Física Médica, Facultad de Medicina, Avda. Menéndez Pidal s/n, Universidad de Córdoba, 14071 Córdoba; [7] Dep. Farmacología, Toxicología y Medicina Legal y Forense, Facultad de Medicina, Avda. Menéndez Pidal, s/n, Universidad de Córdoba, 14071 Córdoba; [8] Laboratorio de Arqueobiología, Avda. Juan Pablo II s/n, Universidad Nacional de Trujillo, 13011 Trujillo (Peru); [9] Centro de Investigaciones Arqueobiológicas y Paleoecológicas Andinas Arqueobios, C/. Martínez de Compañón 430-Bajo 100, Urbanización San Andrés, 13008 Trujillo (Peru); [10] Instituto de Agricultura Sostenible (IAS), Consejo Superior de Investigaciones Científicas (CSIC), Alameda del Obispo s/n, 14080 Córdoba

Abstract

The phenotype is the result of the interaction of the genotype with epigenetic factors and the environment. It has been considered that genetics is responsible for >70% of facial phenotype. Interestingly, besides sequencing nucleic acids, it is possible to generate DNA methylation maps of ancient remains, to determine phenotypes. Other application areas are medicine, forensics and law-enforcement. Thus, breakthroughs in nucleic-acid analyses are allowing to determine the phenotype from genotypic data. That is also being facilitated by developments in software (including parallel processing) and hardware, including neural engines (neural-network hardware). New strategies involving artificial intelligence and machine learning have been also deployed to reach such a goal. Phenotyping is a challenging task, on the edge of current technology. Promising results have already been obtained, including prediction of Neanderthal (*Homo sapiens neanderthalensis*) and Denisovan (*Homo sapiens denisova*) faces. The future is promising in this research area, in which ethical and legal implications should also be considered.

Key words: mutations, DNA, RNA, genome-wide association studies, single-nucleotide polymorphisms, quantitative-trait loci, molecular photofitting, physical appearance, biogeographic ancestry, paleogenomics, paleotranscriptomics.

Resumen

El fenotipo es el resultado de la interacción del genotipo con factores epigenéticos y el medio ambiente. Se ha considerado que la genética es responsable de >70% del fenotipo facial. Curiosamente, además de secuenciar ácidos nucleicos, es posible generar mapas de metilación de ADN de restos antiguos, para determinar fenotipos.

Otras áreas de aplicación son la medicina, ciencia forense y control del cumplimiento de la ley. Así, los avances en el estudio de ácidos nucleicos están permitiendo determinar el fenotipo a partir de datos genotípicos. Ello también se ve facilitado por los desarrollos en software (incluido el procesamiento paralelo) y hardware, incluidos los motores neuronales (hardware de redes neuronales). También se han implementado nuevas estrategias, involucrando inteligencia artificial y aprendizaje automático, para alcanzar dicho objetivo. El fenotipado es una tarea desafiante, a la vanguardia de la tecnología actual. Ya se han obtenido resultados prometedores, incluida la predicción de caras de neandertales (*Homo sapiens neanderthalensis*) y denisovanos (*Homo sapiens denisova*). El futuro es prometedor en esta área de investigación, en la que también se deben considerar las implicaciones éticas y legales.

Palabras clave: mutaciones, ADN, ARN, estudios de asociación del genoma completo, polimorfismos de un solo nucleótido, loci de rasgos cuantitativos, fototipificación molecular, apariencia física, ascendencia biogeográfica, paleogenómica, paleotranscriptómica.

Introduction

The genotype is the genomic composition of biological entities like virusoids, viroids, viruses and cells. It includes: i) main genome; ii) plasmids (mostly in eubacteria prokaryotes, but sometimes also in archaea prokaryotes and eukaryotes); iii) organelle (mitochondria and chloroplasts) genomes in eukaryotes; and iv) plasmids of organelles. The word genotype was coined in 1903 by the Danish botanist Wilhelm Johannsen (Johannsen, 1903). Genes within the genome are unique for haploid cells, but may exhibit the same (homozygous) or different (heterozygous) alleles if two (diploid) or more (polyploid) sets are present within the same cell, or across the species population. August Weismann (1834-1914) noticed that pluricellular organisms may contain somatic cells (that build the body), as well as germ cells (carrying heredity) (Winther, 2001).

On the other hand, observable features of biological entities are called phenotype. The genotype-phenotype duality was proposed by Wilhelm Johannsen (Johannsen, 1911). Yet, the phenotype may not be determined by the genotype alone. Other involved elements may be the environment (which is not inherited) and epigenetic factors, which may be inherited. Therefore, organisms with the same genotype may look or behave differently. On the other hand, organisms with different genotypes may look alike. It has been considered that genetics is responsible for >70% of facial phenotype (Djordjevic et al, 2016). Phenotypes are visible for current biological entities, but may not be available for forensic or ancient samples. Thus, it may be useful to infer phenotypes from the genotypes in such scenarios. This topic is currently in the frontier of knowledge, being actively investigated. There is a wide interest in this area. That includes basic knowledge for studies of both modern and ancient samples, as well as applications in medicine, besides forensics and law-enforcement areas. Indeed, interesting research results have recently been published in this fascinating topic, as described below.

Phenotyping modern intact DNA

Phenotype prediction from genetic information is called phenotyping. That can be accomplished using genotyping data generated with molecular markers, including nucleic-acid sequencing (Scudder et al, 2018), which actually is the ultimate genotyping technology, as we have reviewed (Dorado et al, 2021). Indeed, the First-Generation Sequencing (FGS) represented a revolution, to which we have contributed (Lario et al, 1997), since it allowed to read genetic information for the first time, including the Human Genome Project. The Second-Generation Sequencing (SGS) further improved throughput and reduced cost, allowing to sequence ancient genomes for the first time, as we have reviewed (Dorado et al, 2015). Finally, the Third-Generation Sequencing (TGS) allowed to directly sequence nucleic acids, without previous retrotranscription or amplification. That makes possible to directly sequence ancient RNA (aRNA), as we have reviewed (Dorado et al, 2016; 2020). Phenotyping is also know as molecular photofitting in forensic science, when applied to infer the physical appearance and biogeographic ancestry. But phenotyping is not an easy task. The rationale is that we do not fully understand how genes work and interact with the environment, to produce phenotypes.

To gain knowledge on this research area, significant genetic variants associated with a particular trait can be discovered, using Genome-Wide Association Studies (GWAS). Thus, molecular markers associated to traits of interest can be identified (Fagertun et al, 2015; Kayser, 2015; Marcinska et al, 2015; Wolinsky, 2015; Adhikari et al, 2016; Cole et al, 2016; Roosenboom et al, 2016, 2018; Shaffer et al, 2016; Lee et al, 2017; Tsagkrasoulis et al, 2017; Cha et al, 2018; Claes et al, 2018; Indencleef et al, 2018; Qiao et al, 2018; Richmond et al, 2018; Rolfe et al, 2018; Wang, 2018; Weinberg et al, 2018; Bohringer and DeJong, 2019; Hebbring, 2019; Li et al, 2019; Long et al, 2019; Sero et al, 2019; Wu et al, 2019; Xiong et al, 2019; Balanovska et al, 2020; Pospiech et al, 2020; White et al, 2020; Bonfante et al, 2021; Liu et al, 2021; Naqvi et al, 2021). Among them, Single-Nucleotide Polymorphisms (SNP) can be particularly relevant, since they are usually abundant across genomes. Likewise, Quantitative Trait Loci (QTL) can be useful. Thus, they allow to link molecular markers with quantitative traits in phenotypes. Additionally, mathematical models can be designed to predict phenotypes, from genotypic data.

Recent developments in computing, in general, and bioinformatics, in particular, can be also useful in phenotyping research (DeJong et al, 2018). Among them are multivariate statistical approaches, like Principal-Components Analysis (PCA) (Shui et al, 2017; Crouch et al, 2018), multilevel PCA (mPCA) (Farnell et al, 2020) and toolboxes for integrative analyses (White et al, 2019; Li et al, 2020). Additionally, the term Artificial Intelligence (AI) was coined by John McCarthy in 1956. Thus, AI tries to analyze data and generate results to achieve a particular goal (Legg and Hutter, 2007). Therefore, it mimics human cognitive functions, like learning and problem solving (Russell and Norvig, 2020). On the other hand, the term Machine Learning (ML) was coined by Arthur Samuel in 1959. ML is the part of AI that develops algorithms that can be empirically and automatically improved. This way, the machine is trained with data, gaining new experience to optimize results. Thus,

predictions can be made for new scenarios, that may not have been specifically programmed in advance. That differentiates ML from traditional computing, that only works with pre-programmed algorithms (Alpaydin, 2020; Hu et al, 2020).

Such developments in computing software have been facilitated thanks to hardware improvements, in general, and microprocessors, in particular. Among them are: i) increasing microprocessor clock frequency to generate pulses (clock rate); ii) reducing microprocessor lithographic node; and iii) incrementing the number of cores in multicore (a few) and manycore (high number) of Central-Processing Units (CPU) and Graphics-Processing Units (GPU), allowing parallel processing. Dedicated neural-network hardware is another interesting development. That includes the Neural Engine from manufacturers like Apple <https://www.apple.com>. For instance, the one of the ARM-based Apple Silicon M1 microprocessor is capable of executing 11,000 milliard operations per second, being used for machine learning tasks. Indeed, phenotyping is a multidisciplinary science, including biology, bioinformatics, ethics and law (Claes and Shriver, 2014) (Fig. 1).

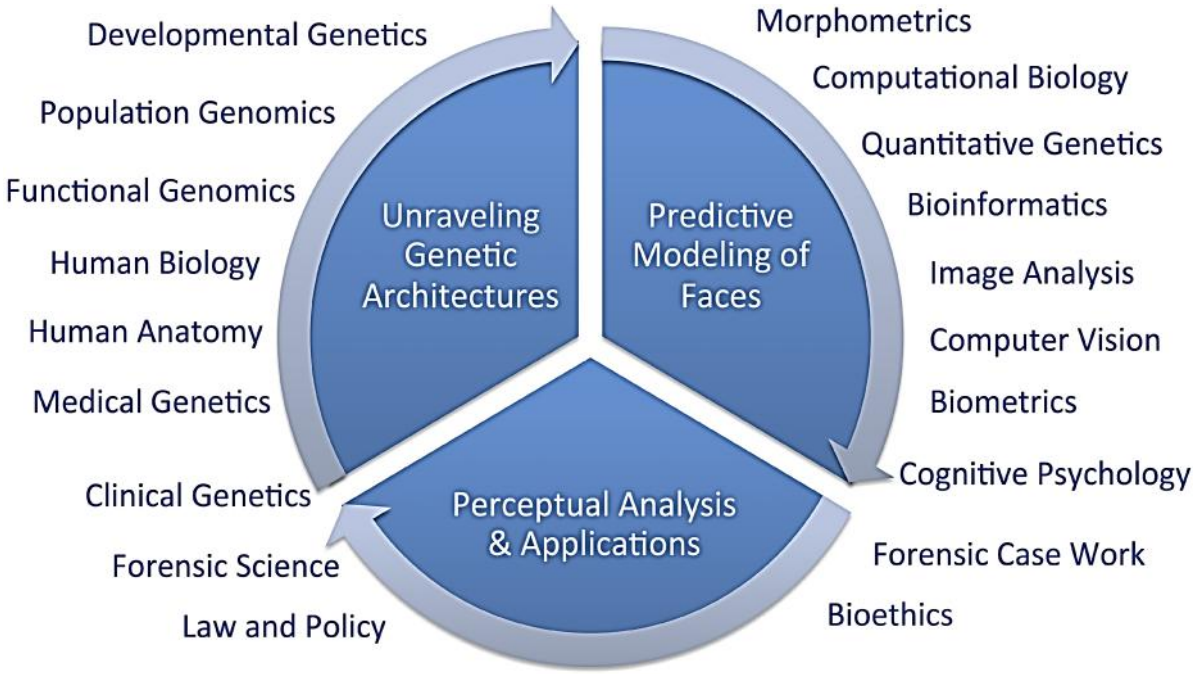

Figure 1.- **Phenotyping framework.** Predicting facial features from DNA data is a multidisciplinary science, involving many knowledge areas. © Public Library of Science (PLoS; Claes and Shriver, 2014).

Some interesting examples of phenotyping have been already published, exploiting such technologies. For instance, just 24 SNP associated to facial variation were used for the first time to infer human faces. They used Bootstrapped Response-based Imputation Modeling (BRIM). As the authors acknowledged, facial prediction

using genotyping data is challenging, but results are promising (Claes et al, 2014a-b). A further step in DNA phenotyping was carried out sequencing whole human genomes, involving the prestigious Craig Venter Institute (Lippert et al, 2017).

Phenotyping forensic and ancient DNA

It is known that the melanocortin 1 receptor (MC1R) is related to pigmentation. Thus, a fragment of the *MC1R* gen from Neanderthal bone remains was amplified, by Polymerase Chain-Reaction (PCR). Interestingly, amplicon sequencing revealed that they contained a mutation producing pale skin and red hair (redhead). It was concluded that at least 1% of homozygous Neanderthals may have had such phenotype (Lalueza-Fox et al, 2007). Since phenotyping modern intact DNA is challenging, much more can be using forensic and ancient DNA (aDNA), which is typically damaged, both physically (short fragments) and chemically (modified nucleotide bases).

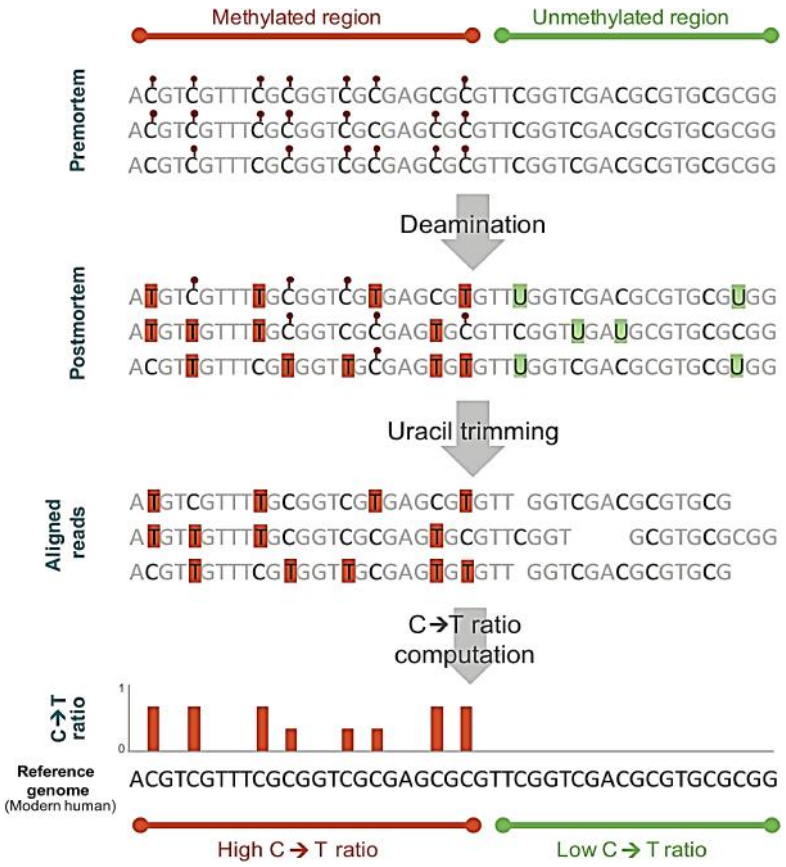

Figure 2.- Identification of aDNA methylation. Premortem DNA may contain methylated and unmethylated cytosines. Postmortem taphonomic scenarios may cause cytosine deamination. Uracil trimming allows to remove such bases in aligned sequencing reads. Finally, C → T transition ratios are used as proxies for reconstructing ancient methylation, aligning to modern reference genomes. © American Association for the Advancement of Science (Gokhman et al, 2014).

A first step to overcome such problems was carried out generating DNA methylation maps of Neanderthal (*Homo sapiens neanderthalensis*) and Denisovan (*Homo sapiens denisova*) remains. Yet, such a goal may not be directly reached, as can be accomplished with modern DNA. Indeed, as said, aDNA may be chemically damaged. Thus, cytosine deamination generates either uracils or thymines (from unmethylated or methylated cytosines, respectively). Uracils can be trimmed, but higher thymine reads are expected in positions with premortem methylated cytosines, as compared to unmethylated positions. Therefore, CpG → TpG transitions are a useful proxy for aDNA methylation in ancient DNA (Gokhman et al, 2014, 2016; Hernando-Herraez et al, 2015; Orlando et al, 2015; Seguin-Orlando et al, 2015; Smith et al, 2015; Hanghoj et al, 2016, 2019) (Fig. 2).

This methodology was further used to infer the skeletal and facial anatomy of Neanderthals and Denisovans. Thus, methylation changes in archaic humans, chimpanzees and modern humans were identified. Gene expression was scored, considering that promoter hypermethylation represses genes. Such downregulation is associated to known mutations causing loss-of-function. Three unidirectional filters allow to predict morphological changes. Skeletal profiles of Neanderthals and chimpanzees were reconstructed, taking into account known morphologies. Furthermore, the accuracy, precision and sensitivity of the method were evaluated (Gokhman et al, 2019) (Fig. 3).

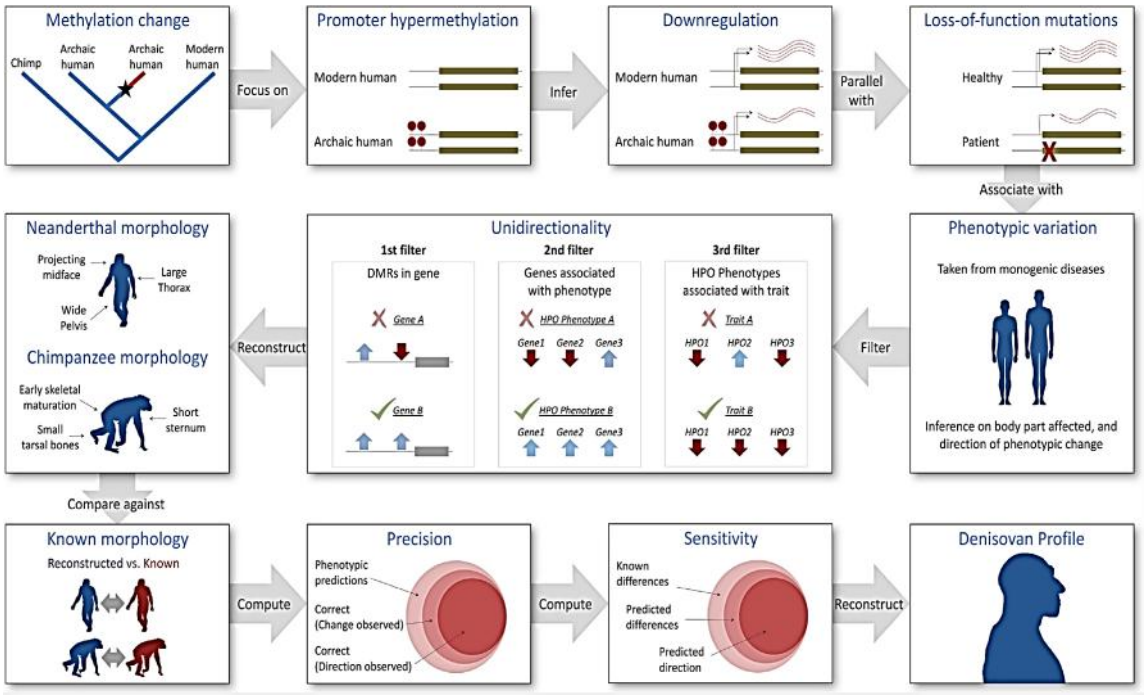

Figure 3.- Workflow to infer Denisovan anatomy from DNA methylation changes. Boxes: 1 to 5) methylation changes were linked to phenotypes; 6) three unidirectionality filters were applied; 7 to 10) accuracy was computed; and 11) Denisovan profile was predicted. © Elsevier (Gokhman et al, 2019).

Interestingly, differential hypermethylation of voice- and face-related genes have also been found between modern humans, when compared to ancient hominids (Neanderthals and Denisovans), as well as modern great apes (chimpanzees). Therefore, it has been proposed that they played a key role in human evolution, shaping our vocal tract and face (Gokhman et al, 2020). DNA methylation patters can also be used to predict age (Zbiec-Piekarska et al, 2015) and diseases like schizophrenia (Banerjee et al, 2018, 2019), as well as gene expression of ancient samples (Batyrev et al, 2019; Hahn et al, 2020; Liu et al, 2020; Mathov et al 2020; Rubi et al, 2020). That has implications for ancient environments and life styles (Gokhman et al 2017). Likewise, gene regulation in modern and archaic samples can be inferred using indirect approaches (Yan and McCoy, 2020) and trained statistical models (Colbran et al, 2019).

On the other hand, self-domestication is defined as a behavioral process involving reduced aggression and increased collaboration, as shown by hominids like bonobos and humans (Wrangham, 2003). Interestingly, molecular biology applied to archaic and modern humans have shown that the Bromodomain Adjacent to Zinc-finger domain 1B (*BAZ1B*) gene was involved in self-domestication, being a master regulator of modern human face (Zanella et al, 2019). Therefore, methodologies involving both archaeology and molecular biology, including paleogenomics, paleotranscriptomics and paleoproteomics, as we have reviewed (Dorado et al, 2007-2014, 2017, 2018, 2019), have allowed to infer faces of archaic hominids, like Neanderthals and Denisovans (Fig 4).

Figure 4.- Prediction of Neanderthal and Denisovan faces. From left to right: artist's reconstruction of adolescent and adult females of such subspecies, respectively. © 2019 Royal Pavilion & Museums; Brighton & Hove (left) and Maayan Harel (right).

Concluding remarks and future prospects

Recent developments in archaeology, molecular biology, software and hardware are allowing to carry out scientific projects that were not previously possible. One of them is to infer faces of modern or archaic humans, from their genomes. This genotypic-based phenotypic prediction is challenging, being in the limit of what is currently possible. Yet, some interesting accomplishments in this area have already been published, with promising results. A more accurate phenotypic prediction should be possible with the optimization of current technologies and development of new ones. Among them are: i) structural genomics, including non-coding DNA and identification of all genes present in genomes; ii) functional genomics, including implications of spurious or generalized transcription, as we have reviewed (Dorado et al, 2020); and iii) epigenetics, including genomic-methylation maps. Finally, the ethical and legal implications of genomic research should be taken into account (Berkman et al, 2016).

***Acknowledgements*.** Supported by "Ministerio de Economía y Competitividad" (MINECO grant BIO2015-64737-R) and "Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria" (MINECO and INIA RF2012-00002-C02-02); "Consejería de Agricultura y Pesca" (041/C/2007, 75/C/2009 and 56/C/2010), "Consejería de Economía, Innovación y Ciencia" (P11-AGR-7322) and "Grupo PAI" (AGR-248) of "Junta de Andalucía"; and "Universidad de Córdoba" ("Ayuda a Grupos"), Spain.

References

Adhikari K, Fuentes-Guajardo M, Quinto-Sánchez M, Mendoza-Revilla J, Camilo Chacón-Duque J, Acuña-Alonzo V, Jaramillo C, Arias W, Lozano RB, Pérez GM, Gómez-Valdés J, Villamil-Ramírez H, Hunemeier T, Ramallo V, Silva de Cerqueira CC, Hurtado M, Villegas V, Granja V, Gallo C, Poletti G, Schuler-Faccini L, Salzano FM, Bortolini MC, Canizales-Quinteros S, Cheeseman M, Rosique J, Bedoya G, Rothhammer F, Headon D, González-José R, Balding D, Ruiz-Linares A (2016): A genome-wide association scan implicates *DCHS2*, *RUNX2*, *GLI3*, *PAX1* and *EDAR* in human facial variation. Nature Communications 7: 11616 (11 pp).

Alpaydin E (2020): "Introduction to Machine Learning" (4th ed). MIT Press (Cambridge, MA, USA).

Balanovska E, Lukianova E, Kagazezheva J, Maurer A, Leybova N, Agdzhoyan A, Gorin I, Petrushenko V, Zhabagin M, Pylev V, Kostryukova E, Balanovsky O (2020): Optimizing the genetic prediction of the eye and hair color for North Eurasian populations. BMC Genomics 21(Suppl 7): 527 (13 pp).

Banerjee N, Polushina T, Bettella F, Giddaluru S, Steen VM, Andreassen OA, Le Hellard S (2018): Recently evolved human-specific methylated regions are enriched in schizophrenia signals. BMC Evolutionary Biology 18: 63 (11 pp).

Banerjee N, Polushina T, Bettella F, Steen VM, Andreassen OA, Le Hellard S (2019): Analysis of differentially methylated regions in great apes and extinct hominids provides support for the evolutionary hypothesis of schizophrenia. Schizophrenia Research 206: 209-216.

Batyrev D, Lapid E, Carmel L, Meshorer E (2019): Predicted archaic 3D genome organization reveals genes related to head and spinal cord separating modern from archaic humans. Cells 9: 48 (9 pp).

Berkman BE, Shapiro ZE , Eckstein L, Pike ER (2016): The ethics of large-scale genomic research. In: Collmann J, Matei SA (eds): "Ethical Reasoning in Big Data: An Exploratory Analysis", 53-69. Springer (New York City, NY, USA).

Bohringer S, DeJong MA (2019): Quantification of facial traits. Frontiers in Genetics. 10: 397 (14 pp).

Bonfante B, Faux P, Navarro N, Mendoza-Revilla J, Dubied M, Montillot C, Wentworth E, Poloni L, Varón-González C, Jones P, Xiong Z, Fuentes-Guajardo M, Palmal S, Chacón-Duque JC, Hurtado M, Villegas V, Granja V, Jaramillo C, Arias W, Barquera R, Everardo-Martínez P, Sánchez-Quinto M, Gómez-Valdés J, Villamil-Ramírez H, Silva de Cerqueira CC, Hünemeier T, Ramallo V, Liu F, Weinberg SM, Shaffer JR, Stergiakouli E, Howe LJ, Hysi PG, Spector TD, Gonzalez-José R, Schüler-Faccini L, Bortolini MC, Acuña-Alonzo V, Canizales-Quinteros S, Gallo C, Poletti G, Bedoya G, Rothhammer F, Thauvin-Robinet C, Faivre L, Costedoat C, Balding D, Cox T, Kayser M, Duplomb L, Yalcin B, Cotney J, Adhikari K, Ruiz-Linares A (2021): A GWAS in Latin Americans identifies novel face shape loci, implicating VPS13B and a Denisovan introgressed region in facial variation. Science Advances 7: eabc6160 (18 pp).

Cha S, Lim JE, Park AY, Do JH, Lee SW, Shin C, Cho NH, Kang JO, Nam JM, Kim JS, Woo KM, Lee SH, Kim JY, Oh B (2018): Identification of five novel genetic loci related to facial morphology by genome-wide association studies. BMC Genomics 19: 481 (17 pp).

Claes P, Hill H, Shriver MD (2014a): Toward DNA-based facial composites: preliminary results and validation. Forensic Science International: Genetics 13: 208-216.

Claes P, Liberton DK, Daniels K, Rosana KM, Quillen EE, Pearson LN, McEvoy B, Bauchet M, Zaidi AA, Yao W, Tang H, Barsh GS, Absher DM, Puts DA, Rocha J, Beleza S, Pereira RW, Baynam G, Suetens P, Vandermeulen D, Wagner JK, Boster JS, Shriver MD (2014b): Modeling 3D facial shape from DNA. PLoS Genetics 10: e1004224 (14 pp).

Claes P, Roosenboom J, White JD, Swigut T, Sero D, Li J, Lee MK, Zaidi A, Mattern BC, Liebowitz C, Pearson L, González T, Leslie EJ, Carlson JC, Orlova E, Suetens P, Vandermeulen D, Feingold E, Marazita ML, Shaffer JR, Wysocka J, Shriver MD, Weinberg SM (2018): Genome-wide mapping of global-to-local genetic effects on human facial shape. Nature Genetics 50: 414-423.

Claes P, Shriver MD (2014): Establishing a multidisciplinary context for modeling 3D facial shape from DNA. PLoS Genetics 10: e1004725 (3 pp).

Colbran LL, Gamazon ER, Zhou D, Evans P, Cox NJ, Capra JA (2019): Inferred divergent gene regulation in archaic hominins reveals potential phenotypic differences. Nature Ecology & Evolution 3: 1598-1606.

Cole JB, Manyama M, Kimwaga E, Mathayo J, Larson JR, Liberton DK, Lukowiak K, Ferrara TM, Riccardi SL, Li M, Mio W, Prochazkova M, Williams T, Li H, Jones KL, Klein OD, Santorico SA, Hallgrimsson B, Spritz RA (2016): Genomewide association study of African children identifies association of *SCHIP1* and *PDE8A* with facial size and shape. PLoS Genetics 12: e1006174. (19 pp).

Crouch DJM, Winney B, Koppen WP, Christmas WJ, Hutnik K, Day T, Meena D, Boumertit A, Hysi P, Nessa A, Spector TD, Kittler J, Bodmer WF (2018): Genetics of the human face: Identification of large-effect single gene variants. Proceedings of the National Academy of Sciences of USA 115: E676-E685.

DeJong MA, Hysi P, Spector T, Niessen W, Koudstaal MJ, Wolvius EB, Kayser M, Bohringer S (2018): Ensemble landmarking of 3D facial surface scans. Scientific Reports 8: 12 (11 pp).

Djordjevic J, Zhurov AI, Richmond S, Visigen Consortium (2016): Genetic and environmental contributions to facial morphological variation: A 3D population-based twin study. PLoS One 11: e0162250 (20 pp).

Dorado G, Gálvez S, Rosales TE, Vásquez VF, Hernández P (2021): Analyzing modern biomolecules: the revolution of nucleic-acid sequencing – Review. Biomolecules (section Molecular Genetics) 11: 1111 (18 pp).

Dorado G, Jiménez I, Rey I, Sánchez-Cañete FJS, Luque F, Morales A, Gálvez M, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2013): Genomics and proteomics in bioarchaeology - Review. Archaeobios 7: 47-63.

Dorado G, Luque F, Pascual P, Jiménez I, Sánchez-Cañete FJS, Pérez-Jiménez M, Raya P, Gálvez M, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2015): Second-generation nucleic-acid sequencing and bioarchaeology - Review. Archaeobios 9: 216-230.

Dorado G, Luque F, Pascual P, Jiménez I, Sánchez-Cañete FJS, Pérez-Jiménez M, Raya P, Sáiz J, Sánchez A, Martín J, Rosales TE, Vásquez VF, Hernández P (2016): Sequencing ancient RNA in bioarchaeology - Review. Archaeobios 10: 103-111.

Dorado G, Luque F, Pascual P, Jiménez I, Sánchez-Cañete FJS, Raya P, Sáiz J, Sánchez A, Rosales TE, Vásquez VF (2017): Clustered Regularly-Interspaced Short-Palindromic Repeats (CRISPR) in bioarchaeology - Review. Archaeobios 11: 179-188.

Dorado G, Luque F, Pascual P, Jiménez I, Sánchez-Cañete FJS, Raya P, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2018): Evolution from first hominids to modern humans: philosophy, bioarchaeology and biology - Review. Archaeobios 12: 69-82

Dorado G, Luque F, Pascual P, Jiménez I, Sánchez-Cañete FJS, Raya P, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2019): Bioarchaeology to bring back scents from extinct plants - Review. Archaeobios 13: 66-75.

Dorado G, Luque F, Pascual P, Jiménez I, Sánchez-Cañete FJS, Raya P, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2020): Implications of non-coding RNA on biology and evolution: from first hominids to modern humans - Review. Archaeobios 14: 107-118.

Dorado G, Rey I, Rosales TE, Sánchez-Cañete FJS, Luque F, Jiménez I, Gálvez M, Sáiz J, Sánchez A, Vásquez VF (2009): Ancient DNA to decipher the domestication of dog (REVIEW). Archaeobios 3: 127-132.

Dorado G, Rey I, Rosales TE, Sánchez-Cañete FJS, Luque F, Jiménez I, Morales A, Gálvez M, Sáiz J, Sánchez A, Hernández P, Vásquez VF (2010): Biological mass extinctions on planet Earth (REVIEW). Archaeobios 4: 53-64.

Dorado G, Rosales TE, Luque F, Sánchez-Cañete FJS, Rey I, Jiménez I, Morales A, Gálvez M, Sáiz J, Sánchez A, Vásquez VF, Hernández P (2011): Ancient nucleic acids from maize - A review. Archaeobios 5: 21-28.

Dorado G, Rosales TE, Luque F, Sánchez-Cañete FJS, Rey I, Jiménez I, Morales A, Gálvez M, Sáiz J, Sánchez A, Vásquez VF, Hernández P (2012): Isotopes in bioarchaeology - Review. Archaeobios 6: 79-91

Dorado G, Sánchez-Cañete FJS, Pascual P, Jiménez I, Luque F, Pérez-Jiménez M, Raya P, Gálvez M, Sáiz J, Sánchez A, Rosales TE, Vásquez VF, Hernández P (2014): Starch genomics and bioarchaeology - Review. Archaeobios 8: 41-50.

Dorado G, Vásquez V, Rey I, Luque F, Jiménez I, Morales A, Gálvez M, Sáiz J, Sánchez A, Hernández P (2008): Sequencing ancient and modern genomes (REVIEW). Archaeobios 2: 75-80.

Dorado G, Vásquez V, Rey I, Vega JL (2007): Archaeology meets Molecular Biology (REVIEW). Archaeobios 1: 1-2.

Fagertun J, Wolffhechel K, Pers TH, Nielsen HB, Gudbjartsson D, Stefansson H, Stefansson K, Paulsen RR, Jarmer H (2015): Predicting facial characteristics from complex polygenic variations. Forensic Science International: Genetics 19: 263-268.

Farnell DJJ, Richmond S, Galloway J, Zhurov AI, Pirttiniemi P, Heikkinen T, Harila V, Matthews H, Claes P (2020): Multilevel principal components analysis of three-dimensional facial growth in adolescents. Computer Methods and Programs in Biomedicine 188:105272 (10 pp).

Gokhman D, Lavi E, Prufer K, Fraga MF, Riancho JA, Kelso J, Paabo S, Meshorer E, Carmel L (2014): Reconstructing the DNA methylation maps of the Neandertal and the Denisovan. Science 344:523-527.

Gokhman D, Malul A, Carmel L (2017): Inferring past environments from ancient epigenomes. Molecular Biology and Evolution 34: 2429-2438.

Gokhman D, Meshorer E, Carmel L (2016): Epigenetics: It's getting old. Past meets future in paleoepigenetics. Trends in Ecology & Evolution 31: 290-300.

Gokhman D, Mishol N, de Manuel M, de Juan D, Shuqrun J, Meshorer E, Marques-Bonet T, Rak Y, Carmel L (2019): Reconstructing Denisovan anatomy using DNA methylation maps. Cell 179:180-192.e10 (24 pp).

Gokhman D, Nissim-Rafinia M, Agranat-Tamir L, Housman G, García-Pérez R, Lizano E, Cheronet O, Mallick S, Nieves-Colón MA, Li H, Alpaslan-Roodenberg S, Novak M, Gu H, Osinski JM, Ferrando-Bernal M, Gelabert P, Lipende I, Mjungu D, Kondova I, Bontrop R, Kullmer O, Weber G, Shahar T, Dvir-Ginzberg M, Faerman M, Quillen EE, Meissner A, Lahav Y, Kandel L, Liebergall M, Prada ME, Vidal JM, Gronostajski RM, Stone AC, Yakir B, Lalueza-Fox C, Pinhasi R, Reich D, Marques-Bonet T, Meshorer E, Carmel L (2020): Differential DNA methylation of vocal and facial anatomy genes in modern humans. Nature Communications 11: 1189 (21 pp).

Hahn EE, Grealy A, Alexander M, Holleley CE (2020): Museum epigenomics: charting the future by unlocking the past. Trends in Ecology & Evolution 35: 295-300.

Hanghoj K, Renaud G, Albrechtsen A, Orlando L (2019): DamMet: ancient methylome mapping accounting for errors, true variants, and post-mortem DNA damage. Gigascience 8: giz025 (6 pp).

Hanghoj K, Seguin-Orlando A, Schubert M, Madsen T, Pedersen JS, Willerslev E, Orlando L (2016): Fast, accurate and automatic ancient nucleosome and methylation maps with epiPALEOMIX. Molecular Biology and Evolution 33: 3284-3298.

Hebbring S (2019): Genomic and phenomic research in the 21st century Trends in Genetics 35: 29-41.

Hernando-Herraez I, Garcia-Perez R, Sharp AJ, Marques-Bonet T (2015): DNA methylation: Insights into human evolution. PLoS Genetics 11: e1005661 (12 pp).

Hu J, Niu H, Carrasco J, Lennox B, Arvin F (2020): Voronoi-based multi-robot autonomous exploration in unknown environments via deep reinforcement learning. IEEE Transactions on Vehicular Technology 69: 14413-14423.

Indencleef K, Roosenboom J, Hoskens H, White JD, Shriver MD, Richmond S, Peeters H, Feingold E, Marazita ML, Shaffer JR, Weinberg SM, Hens G, Claes P (2018): Six *NSCL/P* loci show associations with normal-range craniofacial variation. Frontiers in Genetics 9: 502 (14 pp).

Johannsen W (1903): Om arvelighed i samfund og i rene linier. Oversigt Birdy over Det Kongelige Danske Videnskabernes Selskabs Forhandlingerm 3: 247.370.

Johannsen, W. (1911). The genotype conception of heredity. American Naturalist 45: 129-159.

Kayser M (2015): Forensic DNA phenotyping: predicting human appearance from crime scene material for investigative purposes. Forensic Science International: Genetics 18: 33-48.

Lalueza-Fox C, Rompler H, Caramelli D, Staubert C, Catalano G, Hughes D, Rohland N, Pilli E, Longo L, Condemi S, de la Rasilla M, Fortea J, Rosas A, Stoneking M, Schoneberg T, Bertranpetit J, Hofreiter M (2007): A melanocortin 1 receptor allele suggests varying pigmentation among Neanderthals. Science 318:1453-1455.

Lario A, González A, Dorado G (1997): Automated laser-induced fluorescence DNA sequencing: equalizing signal-to-noise ratios significantly enhances overall performance. Analytical Biochemistry 247: 30-33.

Lee MK, Shaffer JR, Leslie EJ, Orlova E, Carlson JC, Feingold E, Marazita ML, Weinberg SM (2017): Genome-wide association study of facial morphology reveals novel associations with *FREM1* and *PARK2*. PLoS One 12: e0176566 (13 pp).

Legg S, Hutter M (2007): A collection of definitions of intelligence. Frontiers in Artificial Intelligence and Applications 157: 17-24.

Li J, Zarzar TG, White JD, Indencleef K, Hoskens H, Matthews H, Nauwelaers N, Zaidi A, Eller RJ, Herrick N, Günther T, Svensson EM, Jakobsson M, Walsh S, Van Steen K, Shriver MD, Claes P (2020): Robust genome-wide ancestry inference for heterogeneous datasets: illustrated using the 1,000 genome project with 3D facial images. Scientific Reports 10: 11850 (15 pp).

Li Y, Zhao W, Li D, Tao X, Xiong Z, Liu J, Zhang W, Ji A, Tang K, Liu F, Li C (2019): *EDAR*, *LYPLAL1*, *PRDM16*, *PAX3*, *DKK1*, *TNFSF12*, *CACNA2D3*, and *SUPT3H* gene variants influence facial morphology in a Eurasian population. Human Genetics 138: 681-689.

Lippert C, Sabatini R, Maher MC, Kang EY, Lee S, Arikan O, Harley A, Bernal A, Garst P, Lavrenko V, Yocum K, Wong T, Zhu M, Yang WY, Chang C, Lu T, Lee CWH, Hicks B, Ramakrishnan S, Tang H, Xie C, Piper J, Brewerton S, Turpaz Y, Telenti A, Roby RK, Och FJ, Venter JC (2017): Identification of individuals by trait prediction using whole-genome sequencing data. Proceedings of the National Academy of Sciences of USA 114: 10166-10171.

Liu D, Alhazmi N, Matthews H, Lee MK, Li J, Hecht JT, Wehby GL, Moreno LM, Heike CL, Roosenboom J, Feingold E, Marazita ML, Claes P, Liao EC, Weinberg SM, Shaffer JR (2021): Impact of low-frequency coding variants on human facial shape. Scientific Reports 11: 748 (13 pp).

Liu Y, Weyrich LS, Llamas B (2020): More arrows in the ancient DNA quiver: Use of paleoepigenomes and paleomicrobiomes to investigate animal adaptation to environment. Molecular Biology and Evolution 37: 307-319.

Long GS, Hussen M, Dench J, Aris-Brosou S (2019): Identifying genetic determinants of complex phenotypes from whole genome sequence data. BMC Genomics 20: 470 (17 pp).

Marcinska M, Pospiech E, Abidi S, Andersen JD, van den Berge M, Carracedo A, Eduardoff M, Marczakiewicz-Lustig A, Morling N, Sijen T, Skowron M, Sochtig J, Syndercombe-Court D, Weiler N; EUROFORGEN-NoE Consortium, Schneider PM, Ballard D, Borsting C, Parson W, Phillips C, Branicki W (2015): Evaluation of DNA variants associated with androgenetic alopecia and their potential to predict male pattern baldness. PLoS One. 10: e0127852 (18 pp).

Mathov Y, Batyrev D, Meshorer E, Carmel L (2020): Harnessing epigenetics to study human evolution. Current Opinion in Genetics & Development 62: 23-29.

Naqvi S, Sleyp Y, Hoskens H, Indencleef K, Spence JP, Bruffaerts R, Radwan A, Eller RJ, Richmond S, Shriver MD, Shaffer JR, Weinberg SM, Walsh S, Thompson J, Pritchard JK, Sunaert S, Peeters H, Wysocka J, Claes P (2021): Shared heritability of human face and brain shape. Nature Genetics 53: 830-839.

Orlando L, Gilbert MT, Willerslev E (2015): Reconstructing ancient genomes and epigenomes. Nature Reviews Genetics 16: 395-408.

Pospiech E, Kukla-Bartoszek M, Karłowska-Pik J, Zielinski P, Wozniak A, Boron M, Dabrowski M, Zubanska M, Jarosz A, Grzybowski T, Ploski R, Spolnicka M, Branicki W (2020): Exploring the possibility of predicting human head hair greying from DNA using whole-exome and targeted NGS data. BMC Genomics 21: 538 (18 pp).

Qiao L, Yang Y, Fu P, Hu S, Zhou H, Peng S, Tan J, Lu Y, Lou H, Lu D, Wu S, Guo J, Jin L, Guan Y, Wang S, Xu S, Tang K (2018): Genome-wide variants of Eurasian facial shape differentiation and a prospective model of DNA based face prediction. Journal of Genetics and Genomics 45: 419-432.

Richmond S, Howe LJ, Lewis S, Stergiakouli E, Zhurov A (2018): Facial genetics: a brief overview. Frontiers in Genetics 9: 462 (21 pp).

Rolfe S, Lee SI, Shapiro L (2018): Associations between genetic data and quantitative assessment of normal facial asymmetry. Frontiers in Genetics 9: 659 (10 pp).

Roosenboom J, Hens G, Mattern BC, Shriver MD, Claes P (2016): Exploring the underlying genetics of craniofacial morphology through various sources of knowledge. Biomed Research International 2016: 3054578 (9 pp).

Roosenboom J, Indencleef K, Lee MK, Hoskens H, White JD, Liu D, Hecht JT, Wehby GL, Moreno LM, Hodges-Simeon C, Feingold E, Marazita ML, Richmond S, Shriver MD, Claes P, Shaffer JR, Weinberg SM (2018): SNPs associated with testosterone levels influence human facial morphology. Frontiers in Genetics 9: 497 (10 pp).

Rubi TL, Knowles LL, Dantzer B (2020): Museum epigenomics: Characterizing cytosine methylation in historic museum specimens. Molecular Ecology Resources 20: 1161-1170.

Russell SJ, Norvig P (2020): “Artificial Intelligence: A Modern Approach” (4th ed). Pearson (London, UK).

Scudder N, McNevin D, Kelty SF, Walsh SJ, Robertson J (2018): Massively parallel sequencing and the emergence of forensic genomics: Defining the policy and legal issues for law enforcement. Science & Justice 58:153-158.

Seguin-Orlando A, Gamba C, Sarkissian C, Ermini L, Louvel G, Boulygina E, Sokolov A, Nedoluzhko A, Lorenzen ED, Lopez P, McDonald HG, Scott E, Tikhonov A, Stafford TW Jr, Alfarhan AH, Alquraishi SA, Al-Rasheid KAS, Shapiro B, Willerslev E, Prokhortchouk E, Orlando L (2015): Pros and cons of methylation-based enrichment methods for ancient DNA. Scientific Reports 5: 11826 (15 pp).

Sero D, Zaidi A, Li J, White JD, Zarzar TBG, Marazita ML, Weinberg SM, Suetens P, Vandermeulen D, Wagner JK, Shriver MD, Claes P (2019): Facial recognition from DNA using face-to-DNA classifiers. Nature Communications 10: 2557 (12 pp).

Shaffer JR, Orlova E, Lee MK, Leslie EJ, Raffensperger ZD, Heike CL, Cunningham ML, Hecht JT, Kau CH, Nidey NL, Moreno LM, Wehby GL, Murray JC, Laurie CA, Laurie CC, Cole J, Ferrara T, Santorico S, Klein O, Mio W, Feingold E, Hallgrimsson B, Spritz RA, Marazita ML, Weinberg SM (2016): Genome-wide association study reveals multiple loci influencing normal human facial morphology. PLoS Genetics 12:e1006149 (21 pp).

Shui W, Zhou M, Maddock S, He T, Wang X, Deng Q (2017): A PCA-Based method for determining craniofacial relationship and sexual dimorphism of facial shapes. Computers in Biology and Medicine 90: 33-49.

Smith RW, Monroe C, Bolnick DA (2015): Detection of cytosine methylation in ancient DNA from five native american populations using bisulfite sequencing. PLoS One 10: e0125344 (23 pp).

Tsagkrasoulis D, Hysi P, Spector T, Montana G (2017): Heritability maps of human face morphology through large-scale automated three-dimensional phenotyping. Scientific Reports 7:45885 (18 pp).

Wang X (2018): Clinical trans-omics: an integration of clinical phenomes with molecular multiomics. Cell Biology and Toxicology 34:163-166.

Weinberg SM, Cornell R, Leslie EJ (2018): Craniofacial genetics: Where have we been and where are we going? PLoS Genetics 14: e1007438 (8 pp).

Wrangham R (2003): The evolution of cooking. In: Brockman J (ed): “The New Humanists: Science at the Edge”. Sterling Publishing (New York, NY, USA), pp. 99-110.

White JD, Indencleef K, Naqvi S, Eller RJ, Hoskens H, Roosenboom J, Lee MK, Li J, Mohammed J, Richmond S, Quillen EE, Norton HL, Feingold E, Swigut T, Marazita ML, Peeters H, Hens G, Shaffer JR, Wysocka J, Walsh S, Weinberg SM, Shriver MD, Claes P (2020): Insights into the genetic architecture of the human face. Nature Genetics 53: 45-53.

White JD, Ortega-Castrillón A, Matthews H, Zaidi AA, Ekrami O, Snyders J, Fan Y, Penington T, Van Dongen S, Shriver MD, Claes P (2019): MeshMonk: Open-source large-scale intensive 3D phenotyping Scientific Reports 9: 6085 (11 pp).

Winther R (2001): August Weismann on germ-plasm variation. Journal of the History of Biology 34: 517-555.

Wolinsky H (2015): CSI on steroids: DNA-based phenotyping is helping police derive visual information from crime scene samples to aid in the hunt for suspects. EMBO Reports 16: 782-786.

Wu W, Zhai G, Xu Z, Hou B, Liu D, Liu T, Liu W, Ren F (2019): Whole-exome sequencing identified four loci influencing craniofacial morphology in northern Han Chinese. Human Genetics 138: 601-611.

Xiong Z, Dankova G, Howe LJ, Lee MK, Hysi PG, de Jong MA, Zhu G, Adhikari K, Li D, Li Y, Pan B, Feingold E, Marazita ML, Shaffer JR, McAloney K, Xu SH, Jin L, Wang S, de Vrij FM, Lendemeijer B, Richmond S, Zhurov A, Lewis S, Sharp GC, Paternoster L, Thompson H, Gonzalez-Jose R, Bortolini MC, Canizales-Quinteros S, Gallo C, Poletti G, Bedoya G, Rothhammer F, Uitterlinden AG, Ikram MA, Wolvius E, Kushner SA, Nijsten TE, Palstra RT, Boehringer S, Medland SE, Tang K, Ruiz-Linares A, Martin NG, Spector TD, Stergiakouli E, Weinberg SM, Liu F, Kayser M; International Visible Trait Genetics (VisiGen)

Consortium (2019): Novel genetic loci affecting facial shape variation in humans. Elife 8: e49898 (web HTML only).

Yan SM, McCoy RC (2020): Archaic hominin genomics provides a window into gene expression evolution. Current Opinion in Genetics & Development 62: 44-49.

Zanella M, Vitriolo A, Andirko A, Martins PT, Sturm S, O'Rourke T, Laugsch M, Malerba N, Skaros A, Trattaro S, Germain PL, Mihailovic M, Merla G, Rada-Iglesias A, Boeckx C, Testa G (2019): Dosage analysis of the 7q11.23 Williams region identifies BAZ1B as a major human gene patterning the modern human face and underlying self-domestication. Science Advances 5: eaaw7908 (15 pp).

Zbiec-Piekarska R, Spolnicka M, Kupiec T, Parys-Proszek A, Makowska Z, Paleczka A, Kucharczyk K, Ploski R, Branicki W (2015): Development of a forensically useful age prediction method based on DNA methylation analysis. Forensic Science International: Genetics 17: 173-179.