

Structural and functional genomics in Old World camels—where do we stand and where to go

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Implications

- Genome-to-phenome research will boost
- our knowledge on molecular mechanisms underlying adaptive and production phenotypes,
 - whole-genome-enabled animal selection
 - genetic diversity conservation.

Key words: genome, livestock, phenome, polymorphism

Introduction

Understanding the structure and function of genomes is important for investigating interactions among genes, between genes and the environment, and deciphering complex traits. With climate change and increasing human population size, it is more important than ever to conserve biodiversity and to improve efficiency in animal health and production using fewer natural resources. This can be achieved by increasing the diversification of locally adapted (livestock) species and by improving the ability to use genotypes to accurately predict relevant adaptive and production phenotypes (Clark *et al.*, 2020). A high-quality reference genome assembly is a prerequisite to initiate functional genome annotation. Significant progress has been made in this direction by sequencing whole animal genomes, detecting sequence variants, associating them to phenotypic traits, and using genomic variation to select for predicted genetic differences in routinely measured traits. Here, we provide a brief overview of the current situation and future challenges in structural and functional genomics in Old World camels.

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Where Do We Stand in Structural and Functional Genomics in Camels

In Old World camels, *de novo* whole-genome sequencing including shot-gun, long reads, and structural Hi-C mapping was used to build high-quality reference genomes on chromosome levels (Elbers *et al.*, 2019; Ming *et al.*, 2020 and references therein). Multiomic, i.e., transcriptomic and proteomic analyses of dromedary (Alvira-Iraizoz *et al.*, 2021) and Bactrian camel (Wu *et al.*, 2014) kidney tissues complemented and advanced these important first steps towards understanding the structure and function of camelid genomes. Building on these newly available high-quality reference genomes, polymorphism and structural and functional analyses of targeted regions were performed, and initial genome-wide association studies were launched. In the following, we briefly summarize the main structural and functional research areas developed so far in Old World camels, and example studies and corresponding references are presented in Table 1.

Structure and polymorphisms in immune response genes

Major parts of the immunome have been resolved including the major histocompatibility complex (MHC), Natural Killer cell and T-cell receptor genes, and the organization of two immunoglobulin light-chain loci. Comparative analyses of innate and adaptive immune response (IR) genes showed a general low diversity among Old World camels.

Environmental adaptation

Comparative genomics revealed complex pathways related to desert adaptations, including fat and water metabolism, stress responses to heat, aridity, intense ultraviolet radiation, and dust. Genes under potential selection in African and Asian dromedaries were involved in inflammatory responses of bacterial and fungal infections as well as the immune system and the circadian rhythm.

Adaptation to heat stress

Camels have developed adaptive mechanisms to heat stress including biochemical and physiological processes. Molecular

Table 1. Examples of structural and functional genetic and omic studies in Old World camels

Structural or functional genomic area	Main addressed topic	Reference
Genome assembly, annotation, and structure	High-quality chromosome-level Bactrian camel reference genome, immunome	Ming et al. (2020), <i>Mol Ecol Res</i> doi:10.1111/1755-0998.13141
	Improved annotation of dromedary reference genome, immunome	Lado et al. (2020), <i>BMC Genom</i> doi:10.1186/s12864-020-06990-4
	High-quality chromosome-level dromedary reference genome	Elbers et al. (2019), <i>Mol Ecol Res</i> doi:10.1111/1755-0998.13020
	Bactrian camel genome assembly at scaffold level	Wu et al. (2014), <i>Nat Comm</i> doi:10.1038/ncomms6188
	Dromedary genome assembly at scaffold level	Fitak et al. (2016), <i>Mol Ecol Res</i> doi:10.1111/1755-0998.12443
	Bactrian camel genome assembly at scaffold level	Jirimutu et al. (2012), <i>Nat Comm</i> doi:10.1038/ncomms3089
	Radiation hybrid (RH) clones to prepare RH dromedary genome map	Perelman et al. (2018), <i>Sci Rep</i> doi:10.1038/s41598-018-20223-5
Transcriptome, general expression studies	Multiomic analysis of dromedary kidney	Alvira-Iraizoz et al. (2021), <i>Com Biol</i> doi:10.1038/s42003-021-02327-3
	Differential expression of Bactrian camel renal medulla	Wu et al. (2014), <i>Nat Comm</i> doi:10.1038/ncomms6188
	Expressed sequence tags of dromedary	Al-Swailem et al. (2010), <i>PLoS One</i> doi:10.1371/journal.pone.0010720
Polymorphism and structure in immune response genes	Cytotoxic Effector Proteins in camels	Futas et al. (2021), <i>Genes</i> doi:10.3390/genes12020304
	Immune response genes related to Middle East Respiratory Syndrome (MERS) in dromedaries	Lado et al. (2021), <i>Cells</i> doi:10.3390/cells10061291
	Immune response genes related to Crimean-Congo hemorrhagic fever virus infection in dromedaries	Lado et al. (2021), <i>Cells</i> doi:10.3390/cells11010008
	Immunome, structure of Bactrian camel nanobodies	Ming et al. (2020), <i>Mol Ecol Res</i> doi:10.1111/1755-0998.13141
	Comparative immunome diversity in Old World camels	Lado et al. (2020), <i>BMC Genom</i> doi:10.1186/s12864-020-06990-4
	Genome analysis of dromedary T-cell receptor gamma (TRG) locus	Antonacci et al. (2020), <i>Dev Com Imm</i> doi:10.1016/j.dci.2020.103614
	Camel adaptive immune response receptor reservoir	Ciccarese et al. (2019), <i>Front Gen</i> doi:10.3389/fgene.2019.00997
	Natural Killer Cell receptor genes in camels	Futas et al. (2019), <i>Front Gen</i> doi:10.3389/fgene.2019.00620
Major histocompatibility complex in camels		Plasil et al. (2019), <i>HLA</i> doi:10.1111/tan.13510
		Plasil et al. (2019), <i>Cells</i> doi:10.3390/cells8101200
		Plasil et al. (2016), <i>BMC Genom</i> doi:10.1186/s12864-016-2500-1
Environmental adaptation	Differentially selected genes between African and Asian dromedaries	Lado et al. (2020), <i>Commun Bio</i> doi:10.1038/s42003-020-1098-7
	Adaptation to desert environment in Bactrian camels	Wu et al. (2014), <i>Nat Comm</i> doi:10.1038/ncomms6188
Adaptation to heat stress, heat shock proteins	Dromedary as livestock model for heat resistant	Tibary et al. (2020), <i>Theriogen</i> doi:10.1016/j.theriogenology.2020.05.046
	Molecular adaptation to heat stress in dromedaries	Hotter et al. (2019), <i>Front Gen</i> doi:10.3389/fgene.2019.00588
	Thermotolerance of camel somatic cells	Saadelin et al. (2019), <i>J Adv Res</i> doi:10.1016/j.jare.2019.11.009
	Sequence analysis of heat shock protein beta-1 in dromedary	Manee et al. (2017), <i>PLoS One</i> doi:10.1371/journal.pone.0189905
	Sequence and expression of heat shock protein 90-alpha	Saeed et al. (2015), <i>Int J Biol Macromol</i> doi:10.3390/ijms12074214
Genomics pathways selected in domestication	Selection signals during domestication in camels	Fitak et al. (2020), <i>Comm Biol</i> doi:10.1038/s42003-020-1039-5

Table 1. Continued

Structural or functional genomic area	Main addressed topic	Reference
Morphological phenotypes growth, coat color	Genes associated with growth in dromedaries	Sani et al. (2022), <i>Animals</i> doi:10.3390/ani12020184
	Low-density single nucleotide polymorphism (SNP) panel related to growth in Iranian dromedaries	Bitaraf Sani et al. (2021), <i>Sci Rep</i> doi:10.1038/s41598-021-87296-7
	Genes associated with coat color variation in dromedaries	Almathen et al. (2018), <i>J Hered</i> doi:10.1093/jhered/esy024
	Coat color and genetic variation in Nigerian dromedaries	Abdussamad et al. (2015), <i>Live Sci</i> doi:10.1016/j.livsci.2015.07.008
Production phenotypes milk, performance	Alternative splice sites generate bioactive peptides in dromedary milk	Ryskaliyeva et al. (2019), <i>Sci Rep</i> doi:10.1038/s41598-019-41649-5.
	Cryptic splice sites in mRNA for Whey acidic protein (WAP) isoforms in camel milk	Ryskaliyeva et al. (2019), <i>BMC Genet</i> doi:10.1186/s12863-018-0704-x.
	Productions and performance traits in dromedaries	Bahbahani et al. (2019), <i>Front Gen</i> doi:10.3389/fgene.2019.00893.
	Proteomic approaches for complex milk proteins in Old World camels	Ryskaliyeva et al. (2018), <i>PLoS One</i> doi:10.1371/journal.pone.0197026
	Alpha-S1-protein polymorphisms in camel	Erhardt et al. (2016), <i>Trop Anim H Prod</i> doi:10.1007/s11250-016-0997-6.
	Molecular characterization of beta-casein in camels	Pauciullo et al. (2014), <i>Gene</i> doi:10.1016/j.gene.2014.06.055
	Genetic variation in kappa-casein in camels	Pauciullo et al. (2013), <i>Gene</i> doi:10.1016/j.gene.2012.10.083
Sequence and expression of myostatin gene	Myostatin structure, polymorphism, and expression in dromedaries	Favia et al. (2019), <i>Front Gen</i> doi:10.3389/fgene.2019.00502
	Myostatin sequence analysis	Muzzachi et al. (2015), <i>Emir J Food Agr</i> doi:10.9755/ejfa.v27i4.19910
Gut microbiome	Gut microbiome of Bactrian camels at different age	He et al. (2019), <i>Curr Microbiol</i> doi:10.1007/s00284-019-01689-6
	Microbiota in different gastrointestinal segments in Bactrian camels	He et al. (2018), <i>Sci Rep</i> doi:10.1038/s41598-017-18298-7
Other physiological phenotypes	Insulin resistance in Bactrian camels	Guo et al. (2021), <i>Sci Rep</i> doi:10.1038/s41598-021-98234-y
	QTLs for hematological traits in Bactrian camels	Guo et al. (2020), <i>Animals</i> doi:10.3390/ani10010096.
	Comparative genomics of keratin loss in mammals	Erhlich et al. (2019), <i>Sci Rep</i> doi:10.1038/s41598-019-47422-y
	Genetic characterization of the oxytocin-neurophysin I (<i>OXT</i>) gene	Pauciullo et al. (2018), <i>PLoS One</i> doi:10.1371/journal.pone.0195407

aspects involved different heat shock protein expression patterns.

Genomic pathways involved in domestication

Selection signals in camels revealed the neural crest cell and thyroid hormone pathways to be involved in the process of domestication.

Morphological phenotypes, growth, and coat color

Low-density single-nucleotide polymorphism analysis revealed genes related to growth in Iranian dromedaries. Polymorphisms in *MC1R* and *ASIP* genes were associated with coat color variation in dromedaries.

Production phenotypes, milk, and performance

Proteomic approaches resolved the complexity of the milk protein fraction of dromedary, Bactrian camels, and hybrids. Cryptic and alternative splice sites in mRNAs in camel milk were investigated as well as the structure and variation in alpha-, beta-, and kappa-casein genes. Genes related to chondrogenesis, energy balance, and urinary system development were under selection between packing and racing dromedaries.

The *myostatin* gene, a negative regulator of skeletal muscle mass in animals that has a role in determining muscular hypertrophy, was sequenced in camels, and its expression levels in different muscle tissue were established. The *gut microbiome* was characterized by high-throughput sequencing of 16S rRNA in Bactrian camels at different age and gastrointestinal segments.

Finally, *genes and quantitative trait loci (QTLs) related to other physiological functions* were identified involving insulin resistance and hematological traits in Bactrian camels, loss of keratin genes in three evolutionary lineages of mammals (including dromedary), and the oxytocin-neurophysin I (*OXT*) gene with its regulatory regions.

Where Do We Need to Go in Structural and Functional Genomics

To advance structural and functional genomics, that is, genome-to-phenome research in Old World camels, we suggest to follow the path of the Functional Annotation of Animal Genomes (FAANG) project (Clark *et al.*, 2020 and references therein). Focusing on 1) pangenomes and comparative genomics, 2) large-scale genotype-to-expression characterization, 3) large phenotype collections, and 4) germplasm banking will accelerate genome-to-phenome research to improve the use and conservation of Old World camels' genomic diversity in relation with adaptation, production, and sustainability traits. Initial pangenomic studies have been done, with notable examples represented by whole-genome re-sequencing of 105 Asian *Camelus bactrianus* genomes (NCBI-SRA accession number SRP107089) and hundreds of African and Asian *Camelus dromedarius* genomes (Illumina Eleventh Agricultural Greater Good Initiative). While these projects covered major geographic regions/countries and identified millions of single nucleotide polymorphisms (SNPs), amenable to implementation within array-based genotyping platforms, future studies shall focus on in-depth analyses of within-population/within-country genetic diversity, to capture fine-scale genomic variation and population structures. This will assist prioritization of in vitro germplasm conservation of endangered stocks. The availability of medium-density SNP arrays and the perspective for high-density formats will foster more detailed genomic sequence variation catalogs. Further reducing genotyping platform costs will allow affordable technology access to less-developed countries and low-added value industries such as milk and meat commodities in marginal areas.

The wealth of data that are (will be) available for the two domestic Old World camel species deserves further functional and comparative genome mining, also exploiting existing whole-genome sequence collections for the wild counterpart (*Camelus ferus*). This will improve gene annotation, understanding of protein domain architectures and their evolution across taxa, as well as the identification of conserved, or peculiar, regulatory elements and signaling pathways.

Over the last decade, genome wide association study (GWAS) has allowed associating genetic loci to various livestock quantitative traits; similar expectations exist for Old World camels, provided that reliable large-scale phenotyping can successfully be implemented in these species. Notably, this is not trivial under the currently practiced extensive/semi-extensive, highly-mobile farming systems. Understanding the underlying functional mechanisms in camels has been

limited so far, hampering the translation of the associations into action. Studies of expressed QTL (eQTL) enrichment among trait-associated variants have highlighted the importance of gene expression regulation in phenotypic variability. Hence, the next step in transcriptomic studies is to integrate large data from different biological layers, that is, by ex-post integrating eQTL information in GWAS results based on co-localization principles or by ex-ante integrating information whether an SNP regulates the expression of a gene into GWAS. This will increase the power to identify trait-associated loci and provide mechanistic insights. Further large-scale efforts in extending the current Old World camels transcriptome dataset in terms of the number and type of examined tissues and animals under different eco-climatic

About the Authors



Pamela A. Burger graduated in 2004 as a veterinarian at the Vetmeduni Vienna with a special focus on mitochondrial disorders in cheetahs and snow leopards. Immediately afterwards, she started her first project about the genetic origin and domestication of Old World camel. Main questions were to identify the genetic origin and species status of the last wild two-humped camels in

Mongolia and China, and the process of domestication in dromedaries and Bactrian camels. In 2015, she founded the International Camel Consortium for Genomic Improvement and Conservation together with colleagues from Italy, Saudi Arabia, France and Kuwait. In 2021, Pamela Burger acquired her Habilitation (equivalent to Associate Professorship) in 'Animal Genetics and Conservation' and is head of the Population Genetics and Conservation group at the Research Institute of Wildlife Ecology, Vetmeduni Vienna; her current projects investigate the immune genome of camels and felids in response to economical relevant and zoonotic diseases. **Corresponding author:** pamela.burger@vetmeduni.ac.at

Elena Ciani is an Associate Professor at the University of Bari (Italy) in Animal Breeding and Biotechnologies applied to the livestock sector. She is nationally and internationally active in the field of genomic characterization of local livestock resources,



with emphasis on the ovine species, as a tool to orient management and conservation decisions. She has conducted studies on sheep that have contributed to the understanding of the evolutionary processes behind the formation of modern breeds and of the molecular mechanisms underlying phenotypes with adaptive significance (fat-tail) and qualitative characteristics of wool production. It is part of the International Sheep Genomics Consortium. She is involved in several European projects with North-African countries for the sustainable development of the dromedary sector. She is a Founding member of the International Camel Consortium for Genetic Improvement and Conservation; currently, she is a coordinator of an international project funded by the biotech company Illumina for the whole-genome characterization of the dromedary genetic diversity.

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conditions will boost our understanding of the functional consequences of genetic variation.

Particular challenges in Old World camels to achieve research-to-practice goals include 1) the need for data sharing policies/practices, 2) human resources empowerment, 3) a better integrated scientific community and stronger interactions among different stakeholder categories, and finally 4) coordinated national/international investments for successful long-term management of the required infrastructures and facilities for camel research and development.

Conclusion

Old World camels can count now on high-quality reference genome assemblies and hence are ready—from a scientific perspective—to move to a research-to-practice level, while benefiting from advanced “omic” technologies and approaches for better functional annotations of regulatory elements and pathways. This will pave the way to omic-empowered genomic selection, genome-enabled management, and ultimately, to understanding, conserving, and utilizing Old World camels’ genomic diversity. Now, it is primarily the national governments’ turn to promote the establishment of strong public–private partnerships for large-scale, routine phenome characterization and to integrate research results into sustainable breeding management and conservation practices.

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