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Value of animal experiments in drug development for hypertension and associated diseases

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Since the 1960s, more than 150 new drugs for the prevention or treatment of cardiovascular disease (CVD) including blood pressure (BP) lowering drugs have been approved[3]. Nevertheless, recent reports divulge a stagnation in the discovery and development of new cardiovascular therapeutics[3]. This seems problematic against the background that elevated BP remains the leading cause of premature death worldwide and accounts for over 200 million disability-adjusted life years[10]. Recent technological advances for target identification together with the development of novel analytical technologies in animal models for BP phenotypes and hypertension-mediated organ damage (HMOD) could tackle this challenge. Here, we briefly discuss the opportunities provided by animal models to support and improve the discovery and preclinical phase of the drug development process of novel therapeutics in hypertension and HMOD (**Figure 1**).

Recent large genome-wide association studies (GWAS) for BP traits identified >1000 independent genetic loci associated with BP phenotypes and thereby enrich our understanding of the complex, polygenetic nature of BP regulation[4]. This knowledge could potentially impact on risk stratification of patients with genetic risk scores, drug selection by pharmacogenomics, and the discovery of novel molecular entities for drug development (recently reviewed in[7]). So far, however, only a few novel promising candidates emerged among the numerous loci identified. Ironically, UMOD (uromodulin) encoding Tamm-Horsfall protein, that has been known since the 19th century[2] emerged as a valuable new pharmacological target. Subsequent to the identification of a single nucleotide polymorphism (SNP) at the UMOD locus and its association with hypertension in early GWAS, gene targeting of UMOD in mice strongly supported a functional role of UMOD for BP regulation and salt-sensitivity[7]. Thus, the `UMOD example´ represents a case in point by highlighting the importance of experimental animal work in complementing GWAS studies (**Figure 1**).

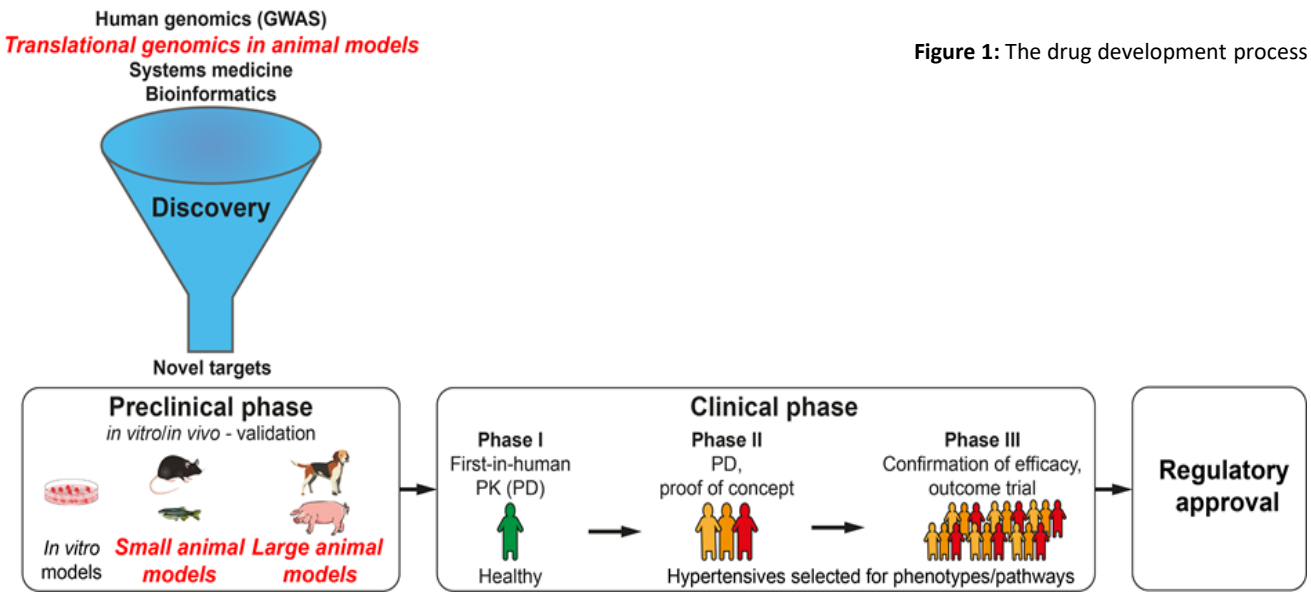

Figure 1: The drug development process

In addition, translational genomic work-up in inbred rodent models of hypertension and HMOD represents another powerful strategy to identify novel targets for therapeutic development[1,7]. These inbred models with their homogeneous genomic background allow tightly controlled experiments to analyze genotype – phenotype relations without the confounding heterogeneous genetic background present in human large – population GWAS[6,8].

A recent study demonstrated that the integrative analysis of the genomic architecture of quantitative trait loci (QTL) identified in inbred hypertensive rat models is a powerful strategy to identify novel targets for HMOD in the kidney (Figure 2)[9]. This particular study focused on the analysis of an albuminuria – QTL identified in the Munich Wistar Frömter (MWF) rat strain as a suitable inbred model for HMOD in the kidney. Congenic substitution mapping of the albuminuria locus combined with next generation sequencing and compartment – specific RNA sequencing analysis in isolated glomeruli tissue led to the identification of Tmem63c as a novel target responsible for the onset of albuminuria in the MWF rat strain. Subsequently, the functional role of Tmem63c was evaluated in zebrafish models as another simple in vivo vertebrate animal model. Reduction of tmem63c levels by targeting an ortholog of the gene with CRISPR–Cas9 or Morpholino gene-editing technologies, respectively in developing zebrafish embryos lead to compromised functionality of the glomerular filtration barrier. The albuminuria - like phenotype could be readily analyzed using transgenic reporter lines; its functional and structural correlates were directly analyzed in the target tissue by imaging analysis using confocal and electron microscopy (**Figure 2**). The potential translational relevance of TMEM63C for kidney damage in humans was confirmed in human biopsies of patients with corresponding kidney disease phenotypes by showing a loss of glomerular TMEM63c in podocytes of patient samples[9].

Taken together, the `UMOD example´ clearly indicates the power of the reverse genetics approach in animals models to validate the functional role of a potential candidate detected in GWAS in humans; in this case UMOD as a candidate for BP regulation and potential target for BP lowering therapy. Conversely, the `Tmem63c example´, supports the power of the forward genetics approach by starting with the analysis of the phenotype in animal model, leading to the discovery of a novel candidate gene for validation in humans studies.

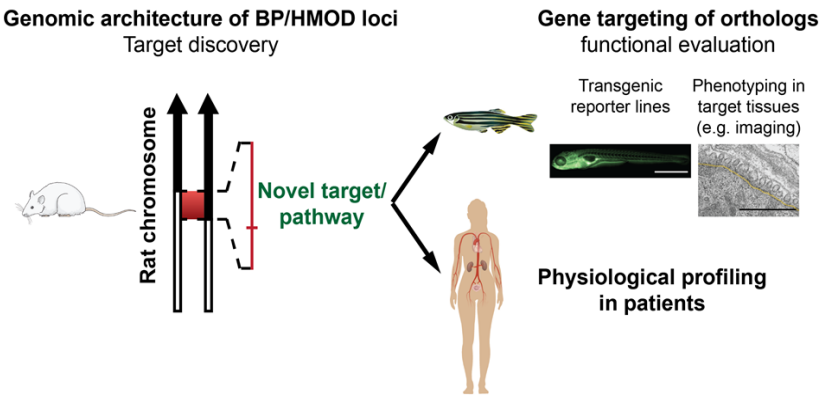

Figure 2: The forward genetics approach: Translational genomics in inbred rodent models as a powerful tool in target identification

Recently, enormous progress has been achieved not only towards the identification of the genetic basis of BP regulation in humans by large-scale GWAS meta-analysis but also by profound advances in systems biology, bioinformatics and computer - aided drug design and development. In addition, major advances in organ–on–a–chip and organoid engineering approaches have been made[5]. Taken together these promising developments led some authors to believe that animal studies can largely be dispensed with in the drug discovery and development process[6].

However, BP and HMOD phenotypes are highly complex traits that depend not only on >1000 genes, but also on environmental conditions with interactions among genes, environment and age. Clearly, the systematic in depth analysis of this complexity in human studies alone has its limitation[6]. Consequently, the combination of observations in humans with the power of experiments in animal models in a virtuous cycle still seems to be an appropriate and promising approach to support drug development in hypertension and associated diseases (Figure 3). In conclusion, despite recent advances in genomics, systems biology, bioinformatics and computer -aided drug design and development, studies in animal models such as rodent models with hypertension and HMOD are still important and indispensable for drug discovery and development.

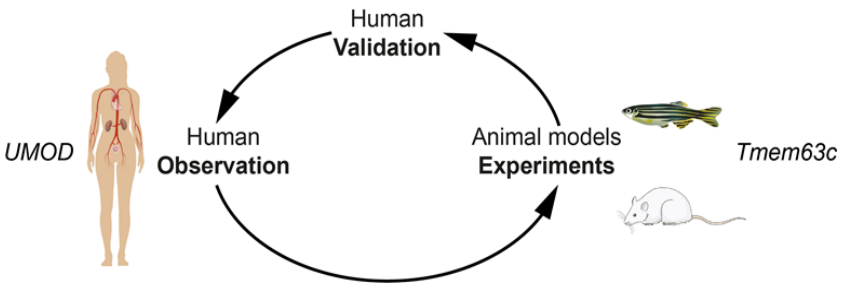

Figure 3: The virtuous cycle of observations in humans and animal models (adapted from (Nadeau & Auwerx, 2019).

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