



# 20 Years of Hypertension Research Using Genetically Modified Animals: No Clinically Promising Approaches in Sight

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## Summary

The incidence of essential or primary hypertension is increasing, especially in the northern hemisphere, but although the disease displays clear symptoms, its aetiology appears very complex, and thus no causal treatment is available yet. In the 1990's, genetically modified animals (GMO) were considered to be the key to solving this problem of high complexity. However, until now, although a few approaches have shown that old, well-known drugs have a positive effect (decrease of blood pressure) on such animal models of hypertension, no approach has appeared in the literature of this area of research which might indicate a direct connection between GMO and a therapeutic strategy to treat or prevent this type of hypertension in humans. Instead, criticism of the GMO approach has accumulated in the last years, arguing that it is misleading as this disease does not have a monogenic cause and so complementary regulatory mechanisms could prevent the true identification of the function of the modified genes. Furthermore, the technology is best developed in mice, whose physiology of blood pressure is different from that of humans. Because of species specificity, it is not easy to extrapolate the results from animal models of hypertension to human hypertension. Also, in the years 2000 to 2004 a reorientation of the technology and the aims of this kind of research took place. Therefore, although these approaches are without exception deemed "very promising" in the literature, it cannot be expected that research on GMO will make any contribution to a new therapeutic strategy in the near future.

**Keywords:** primary hypertension, genetically modified animals-GMO, diagnostic and therapeutic application, target molecules

## 1 Introduction

Blood pressure of at least 140 mm Hg systolic and 90 mm Hg diastolic is the hallmark of arterial hypertension. The cause is only known in about 10% of patients: renal disease, tumours, drugs or diabetes. This so called secondary hypertension may normalise when the underlying disease is treated. In about 90% of patients, no organic cause for the increased blood pressure can be identified, and these cases are termed primary or essential hypertension. This is a multifactorial disease in which genetic and environmental factors interact in a complex way. The consequences of untreated hypertension are arteriosclerosis, coronary heart disease, heart attack, heart failure, renal failure, arterial occlusion and stroke.

Blood pressure is regulated by many physiological systems that have pleiotropic effects and interact in a complex way, including the renin-angiotensin-aldosterone system (RAAS), the

vegetative nervous system, the kinin-kallikrein-system (KKS), the vascular endothelium, natriuretic peptides, and dopamine receptors. Many genes that may influence blood pressure have been identified by systematic analysis of the human genome and by using animal models (Agarwal et al. 2005; Marteau et al., 2005; Mullins et al., 2006). However, the underlying molecular mechanism that leads to the development of primary hypertension is not yet clear. Almost all results and therapeutic approaches up until now have been gained from *in vitro* experiments and animal models for hypertension research, e.g. the spontaneously hypertensive rat, the Milan rat and the Dahl rat. These animal models were selected because of their hypertonic phenotype, which may have complex causes.

Genetic manipulation offers a complementary strategy, allowing one gene to be selectively overexpressed (transgene technology), underexpressed (knock down technology) or turned off (knock out technology), either in selected tissues or in all the

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tissues that normally express it. The knock out technology is easier to apply in mouse than in rat (Tesson et al., 2005). The knock down technology (RNAi) has been employed successfully in both mice and rats (melo et al., 2005).

Insights gained from such animal experiments should help us understand the interactions between the blood pressure regulating systems. With the help of GMO the researchers also aim to identify new, therapeutically relevant targets (e.g. receptors or pharmacologically susceptible regions on the DNA or on proteins), as most hypertensive patients cannot control their blood pressure with the currently available drugs (Scott et al., 2006).

This study aims to investigate whether and in what quantity and quality, the last 20 years of research with GMO have been able to establish, introduce or support new therapies for human primary hypertension.

## 2 Methods

In this study, we searched PubMed for literature indicating a connection between hypertension and transgenic and knock out animal models. For this, all reviews in English between 2000 and June 2007 that included the following key words were assessed: "hypertension knock out animal", "hypertension transgenic animal", "hypertension knock down animal", "hypertension overexpressed animal". Finally all relevant subheadings (see Results sections: A I-IV) were combined with these terms. All articles (reviews and experimental reports) with indications of clinical relevance in the abstract were considered, including those that described suggestions for therapies for the sequelae of hypertension. Articles with the following contents were excluded: limited connection to hypertension (e.g. glaucoma, alcoholic cardiomyopathy), possible improvements but not therapies of the consequences of hypertension, other diseases in which hypertension only plays a secondary role (e.g. obesity, diabetes, renal disease, arteriosclerosis, metabolic syndrome, menopause, pregnancy, drug use), other forms of hypertension (e.g. pulmonary hypertension, portal hypertension, monogenic hypertension, malignant hypertension), which are entirely unrelated to primary hypertension. The approach is described in detail in our original study, commissioned by the Fund for Animal-free Research in Zurich and presented to that organisation in

the form of a detailed report. All articles that were found by this search strategy, which reported on transgenic and knock out animal models and included possible references to humans in their abstracts were analysed. Those publications that contained a reference to a possible therapeutic strategy in humans or that contained analogies to humans were analysed more thoroughly by reading the entire study. Where no references to humans were found or the articles dealt only with basic research, the reports were not further analysed.

Beside the references to humans, the importance of a publication was deemed to correlate with the frequency of its citation in the scientific literature. Hence, citation frequencies for all publications within our search strategy were retrieved.

## 3 Results

The PubMed search resulted in a total of 577 reviews. Of these, 115 reviews published between 2000 and June 2007. The abstracts of these reviews were analysed to determine whether they described a connection between transgenic or knock out animals and primary hypertension. Reviews from animal experiments before 2000 were not considered. At this time, a reorientation occurred with regard to such animal experiments, so that earlier publications and their citations have become practically meaningless. The selected reviews were categorised according to their research focus. 56% of the articles dealt with basic research (no evidence of possible applications of the results), 44% with applied research (possible applications of the results for clinical use) and 0% with clinically orientated research (direct applications of the results from GMO to humans). The number of citations and the citation index (citation frequency / time) were determined for these reviews and are summarised in Table 1. There were no apparent trends regarding the research content or emphasis.

The PubMed search was then expanded by one search term (A I-IV with subitems) to categorise all relevant sources (reviews and original articles) between 2004 and 2007 regarding primary hypertension and GMO. All known physiologically active blood pressure regulating systems were considered. This search delivered 349 articles with regard to primary hypertension. 78% dealt with basic research, 22% focused on applied

Tab. 1: Summary of the reviews with citation number and citation index\*

Reviews n = 115	Citation index 0 (no citations)	Citation index 0.1-0.7 (1 to 5 citations)	Citation index 0.8-1.4 (6 to 10 citations)	Citation index more than 1.4 (more than 10 citations)
mainly applied research n = 66	21	4	9	32
mainly basic research n = 49	19	6	5	19

\* The index was calculated as the number of citations divided by the time between year of publication (up to 2000) and 2007. The results were divided into 4 categories, from citation index 0 (0 citations in a maximum of 7 years) to more than 1.4 (10 citations in a maximum of 7 years). At least one citation per year was required to achieve a citation index of 1.

research, but none described direct applications of the results gained with GMO in humans.

All retrieved publications with connections between primary hypertension and GMO (reviews and original articles) were considered for the following arrangement of the results.

### **A) The blood pressure regulating systems and their role in hypertension – possible targets and their clinical relevance**

- I) The vegetative nervous system
1. Heart  $\beta$ -1-adrenergic receptors ( $\beta$ -1 AR), respectively adrenoreceptor kinases (ARK), heart M 2-receptors, atrial natriuretic peptide (ANP)
  2. Vascular adrenoreceptors:  $\alpha$ -adrenoreceptors ( $\alpha$ -AR),  $\alpha$ -2-adrenoreceptors ( $\alpha$ -2 AR)
  3. Renal adrenoreceptors: renal  $\beta$ -2-adrenoreceptors ( $\beta$ -2 AR).
- II) The vascular endothelium
1. Prostanoids, cyclooxygenases (COX), receptor subtype EP2 for prostaglandin E2 (PGE2 EP2)
  2. Nitric oxide (NO), endothelial NO synthase (eNOS)
  3. Peptides: natriuretic peptide (NP), atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP); NP-receptors and neutral endopeptidase (NEP); angiotensin II (ANG II); endothelin (ET-1), endothelin-1 receptors (ET-A and ET-B), endothelin converting enzyme (ECE); urotensin II (U II); endothelium-derived hyperpolarising factor (EDHF).
- III) The Renin-Angiotensin-System (RAS)
1. Renin
  2. Renin-Angiotensin-System (RAS), angiotensin converting enzyme (ACE), angiotensin converting enzyme 2 (ACE2), angiotensin II-receptor 1 (AT1), angiotensin II-receptor 2 (AT2).
- IV) Other targets
- Angiotensin IV (Ang IV), adrenomedullin, asymmetric dimethylarginine (ADMA), hemoxygenase 1 (HO-1), regulator of G protein signaling 2 (RGS 2), chromogranin (CHGA), vasodilatory neuropeptides as calcitonin gene-related peptide (CGRP) und substance P (SP), telomerase, neuropeptide Y receptor Y1 (NPY Y1), vascular  $\text{Na}^+/\text{Ca}^{2+}$  exchanger type 1 (NCX1),  $\text{Na}^+/\text{K}^+$  ATPase, reactive oxygen species (ROS), dopamine receptors, peroxisome proliferator activated receptor gamma (PPARgamma), PI3K gamma phosphoinositide-3 kinase gamma (PI3Kgamma), kallikrein kinin system (KKS), connexin, nonphagocytical NADPH oxidase, adenosine receptors, superoxide dismutase (SOD), RhoA/Rho-kinase (ROCK), bone morphogenic protein 4 (BMP4), cytochrome P450 (CYP450)/lipoxygenase (LOX), tachykinine.

A detailed discussion of all publications found using these search terms would exceed the scope of this publication. In the complete study, further physiologically relevant connections to the various search terms are described in detail. A copy of the complete study may be requested from the Fund for Animal-free Research in Zurich, Switzerland.

In summary, none of the publications gave indications for new therapeutic approaches to the treatment of hypertension. Many of the physiological receptors and substances named under AI-IV still have no clinical relevance for the treatment of primary hypertension, i.e. ARK (Davidson and Koch, 2001; Koch, 2002; Petrofski and Koch, 2003; Keys and Koch, 2004; Hata et al., 2004; Hansen et al., 2006; Metaye et al., 2005 and 2006), PGE2 EP2 (Ushikubi et al., 2000; Breyer MD, 2000; Breyer et al., 2000), iNOS (Zhang et al., 2007; Kamanaka et al., 2003; Shen et al., 2003; Perdicakis et al., 2005), ACE2 (Der Sarkissian et al., 2006), AT-2 (Li et al., 2007), Angiotensin IV (Vauquelin et al., 2002; Banegas et al., 2006), adrenomedullin (Beltowski and Jamroz, 2004; Shimosawa und Fujita, 2005; Garcia et al., 2006), ADMA/DDAH (Cooke, 2005), Bach1 (Ozono, 2006), RGS2 (Le and Coffman, 2003; Tang et al., 2003; Doggrell, 2004; Semplicini et al., 2006), CHGA (Kim and Loh, 2005; Rao et al., 2007), CGRP and SP (Watson et al., 2002; Deng and Li, 2005, Marquez-Rodas et al., 2006), telomerase (Serrano and Andres, 2004), NPY Y1 (Pedrazzini, 2004), NCX1 (Iwamoto, 2006), dopamine receptors (O'Connell and Aherne, 2000; Yang et al., 2004; Zeng et al., 2004; Zeng et al., 2006), PPAR-gamma (Halabi and Sigmund, 2005; Touyz and Schiffrin, 2006), ROS (Wilcox, 2005; Touyz, 2004; Suzuki et al., 2006), PI3Kgamma (Oudit et al., 2004; Hirsch et al., 2006), KKS (Katori and Majima, 2003; Marcondes and Antunes, 2005; Chao and Chao, 2005; Chao et al., 2006), connexins (Haefliger et al., 2004), NADPH oxidase (Landmesser et al., 2002, Cogolludo et al., 2005), adenosine receptor (Funakoshi et al., 2006), SOD (Wilcox, 2006), BMP4 (Miriya et al., 2006), CYP450/LOX (Immig 2006, Imming et al., 2007), tachykinine (Walsh and McWilliams; 2006).

For other known targets there are no indications of new therapeutic strategies e.g.,  $\alpha$ -AR (Kable et al., 2000; Kanagy, 2005; O'Connell et al., 2006),  $\beta$ -2 AR (Bray and Boerwinkle, 2000; Guimaraes and Moura, 2001; Queen and Ferro, 2006), COX (Imming et al., 2007), NP (Melo et al., 2000; Schillinger et al., 2005), NEP (Mukkaddam-Daher, 2006), ET-1 (Kohan, 2006; Bolli et al., 2004; Cheng et al., 2004; Muller et al., 2003), AT-1 (Nickenig et al., 2006), HO-1 (Wu et al., 2005).

Further, the clinical relevance of already known target molecules could be confirmed in GMO models of hypertension e.g. NEP (Moro and Berlan, 2006), ET-1 (Chatziantoniou and Dussaule, 2000), ECE (Davenport and Maguire, 2006), renin (Muller and Luft, 2006), ACE (Niu et al., 2002), AT-1 (Crowley et al., 2004), adrenomedullin (Eto, 2001; Shimosawa et al., 2003), HO-1 (Agarwal and Nick, 2000; Hill-Kapturkzac et al., 2002; Chen, 2003; Perrella and Yet, 2003; Nascimiento-Silva et al., 2005; Deshane et al., 2005; Kirkby and Adin, 2006),  $\text{Na}^+/\text{K}^+$ ATPase (Blaustein et al., 2006), ROCK (Cogolludo et al., 2005). In other cases the clinical relevance in humans couldn't be confirmed in such animal models of hypertension e.g., COX (Harris et al., 2004; Mullins et al., 2006; Opay et al., 2006), Urotensin II (Zhu et al., 2006).

### **B) General overview of animal models for human hypertension**

Of 19 reviews describing animal models of hypertension, 11 reviews (58%) listed animal models for cardiovascular diseases. Among these were knock out and transgenic models for the



well-known blood pressure regulating systems; however, the meaning of these animal models for the development of a therapy against the human disease was only speculative in terms like “might be a promising model for the future” or “requires further research” or similar phrases (Audoloy et al., 2000; Bader et al., 2000; Lake-Bruse and Sigmund, 2000; Fukamizu, 2000; Cvetkovic and Sigmund, 2000; Yagil and Yagil, 2001; Sugiyama et al., 2001; Lavoie et al., 2004; Barbosa et al., 2005; Lerman et al., 2005; McBride et al., 2006).

The remaining 8 reviews (42%) were more critical and emphasised the limitations of the animal models when used to try to understand and treat human hypertension (Kurihara, 2001; Mullins and Mullins, 2003; Takahashi and Smithies, 2004; Herrera and Ruiz-Opazo, 2005; Smithies, 2005; Bernstein et al., 2005; Mullins et al., 2006).

Table 2 provides an overview of all the reviews that describe animal models for hypertension and shows the citation index as a measure of the citation frequency. 58% of the reviews were cited on average 0.7 times per year; 42% of the reviews 1.9 times per year.

Furthermore, it becomes obvious that reviews which deal with applied research or at least make sensible suggestions regarding the improvement of research, are cited on average more often than the other reviews. This is different from other literature searches, where basic research is usually cited more often (see for instance: Lindl et al., 2005).

The retrieved abstracts of original publications (except reviews) described animal models for hypertension (Denton et al., 2006; Tesson et al., 2005; Kapoor and Dikshit, 2005), but contained no indications of new therapies for hypertension. A further, more recent search of PubMed for all articles from 2007 until April 2008 also did not show any clinically relevant publications.

### C) Therapeutic approaches for human hypertension

The clinical relevance of GMO was summarised in 2003 by Zambrowicz and Sands. The review describes the well-established targets ACE and AT-1 receptor as if they had been discovered recently in experiments with GMO. However, the clinical relevance of the drugs that were already known was merely confirmed by those experiments.

In 2007, Israili and colleagues suggested that hypertensive patients should first change their lifestyle before submitting to treatment or should at least support their therapy by reducing their risk factors (obesity, excessive salt and fat intake, low potassium diet, lack of physical exercise, excessive alcohol consumption). The new antihypertensive drugs include beta blockers, renin inhibitors, endothelin receptor antagonists, endopeptidase inhibitors – which have already been mentioned in this review, and aldosterone receptor blockers like spironolactone and epleron (Israili et al., 2007). The antihypertensive effects of these mineralocorticoid receptor blockers were discovered long before genetic manipulation (Sturtevant, 1958), though their use is limited by undesirable side effects. Clinical studies with epleron, a new, selective aldosterone receptor antagonist, appear promising (Sierra and Ruilope, 2004).

Based on the positive effects of combination therapy of antihypertensive medications, such as statins and lipid lowering drugs, e.g. calcium channel blockers (Escames et al., 2004; Martin-Ventura et al., 2006), the introduction of a “single pill” was suggested (Cogolludo et al., 2005; Blank et al., 2007).

Preclinical studies with a triple vasopeptidase inhibitor, CGS 35601, which may represent a new antihypertensive drug, have already been performed (Battistini et al., 2006). By inhibiting ACE and ECE, the production of the two strong vasoconstrictor peptides angiotensin II and endothelin is prevented. Furthermore, NEP is inhibited, and thus the degradation of the vasodilatory peptides, such as bradykinin, natriuretic peptides and adrenomedullin, is prevented. However, the idea to inhibit these target molecules did not arise from transgenic or knock-out animal experiments.

Despite positive findings in preclinical studies, gene therapy has not yet gained clinical relevance for the treatment of hypertension (Gelband et al., 2000; Phillips, 2000; Phillips et al., 2000; Khurana et al., 2001; Phillips, 2001; Baker, 2002; Hirooka et al., 2003; Veerasingham et al., 2004; Melo et al., 2004). The analysed publications mention or describe the use of vectors for therapeutic gene transfer that are already in clinical use. These were some small clinical studies for other cardiovascular diseases that had already been completed (Abraham et al., 2007; Quarck and Holvoet, 2004). However, these experiments were not designed carefully enough and are thus without further meaning for future gene therapy (see below). With regard to hy-

**Tab. 2: Summary of the reviews describing animal models of hypertension with citation number and citation index\***

Reviews n = 19	Mean citation index	Citation index 0 (no citations)	Citation index 0.1-0.7 (1 to 5 citations)	Citation index 0.8-1.4 (6 to 10 citations)	Citation index more than 1.4 (more than 10 citations)
mainly basic research n = 11	0.7	5	2	1	3
Mainly applied research n = 8	1.9	3	1	1	4

\* The index was calculated as the number of citations divided by the time between year of publication (up to 2000) and 2007. The results were divided into 4 categories from citation index 0 (0 citation in maximal 7 years) to more than 1.4 (10 citations in maximal 7 years). At least one citation per year is required to achieve a citation index of 1.

hypertension, no clinical studies for any kind of antihypertensive gene therapy have been reported (Melo et al., 2005). The authors of these review claimed that gene therapy for hypertension would be simple and safe. However, it is clear that all these vectors are still not safe enough to be used in humans: cytotoxicity is a risk factor for all vectors and, in addition, cancer and mutations are risks for all viral vectors (Raizada and Der Sarkissian, 2005; Heistad, 2006). Gene therapy via gene transfer using viral or nonviral vectors has only been performed in animal experiments to date (Simonson et al., 2005; Nakamura et al., 2006). With regard to primary hypertension, such approaches still have no discernable clinical impact (Puddu, 2006). Other therapies, such as RNAi or stem cell therapy, are still at the stage of basic research (Melo et al., 2005; Nordlie et al., 2006).

Furthermore, it has been suggested that genetic polymorphisms should be considered in future when deciding on a therapeutic strategy (Siest et al., 2004), although the meaning of polymorphisms for hypertension remains “small and clinically irrelevant” (Roszkopf et al., 2007).

#### **D) Suggestions for the therapy of sequelae of human hypertension**

In animal models, certain targets, such as ARK, endothelin or adrenomedullin, had a positive influence on organ damage resulting from hypertension; however, respective gene therapies are not yet applied in humans.

In connection with cardiac insufficiency – one of the consequences of chronic hypertension – gene therapy led to a reduction of cardiac hypertrophy in animal models (Pleger et al., 2005), but has not yet gained clinical relevance for human therapy.

Furthermore, well-known drugs, such as aliskiren (renin inhibitor, Pilz et al., 2005), imatinib (drug used to treat leukaemia, Schellings et al., 2006), angiotensin II receptor blocker (Yasunari et al., 2005) as well as lipid lowering drugs (e.g., fibrates, Muller et al., 2004), reduce organ damage in animal models.

The antioxidant tetrahydrobiopterin BH<sub>4</sub>, a cofactor of eNOS, has been suggested as a therapy for the consequences of hypertension on the heart (Takimoto et al., 2005; Moens and Kass, 2006). Although oxidative stress (i.e. ROS) seems to be associated with hypertension, therapeutic approaches using antioxidants have been disappointing (Wilcox, 2006).

Regenerative stem cell therapy has been suggested as another promising therapeutic approach to treat the effects of hypertension on the endothelium (Wang et al., 2005). This would involve using endothelial stem cells from bone marrow, which are already in clinical trials (Dong and Goldschmidt-Clermont, 2007). However, regenerative stem cell therapy is entirely unrelated to GMO experiments.

Indications for gene therapy for other cardiovascular diseases have been found, e.g. clinical studies with antisense oligonucleotides against atherosclerosis (“E2F decoy” currently in Phase II/III clinical trials, Aboul-Fadl, 2005), as well as gene therapy based on adenoviral and plasmid-mediated VEGF gene transfer as a therapy for arteriosclerotic cardiovascular diseases (Freedman, 2002; Malecki, 2005). Large clinical studies with gene therapeutic approaches for the sequelae of hypertension (“an-

giogenetic gene therapy”) however did not show the expected positive results observed in small clinical studies and animal experiments (Khurana et al., 2005; Nordlie et al., 2006).

#### **E) Suggestions to improve cardiovascular research**

Despite intensive research, the validation of new therapeutic target molecules is progressing slowly; a target molecule can be validated by developing a drug that selectively interacts with the target molecule and shows clinical effectiveness (Williams, 2003). Many publications contain suggestions or speculations to improve drug development.

Species differences, as well as compensatory mechanisms which attempt to normalise blood pressure despite genetic modification designed to achieve hypertension, complicate the interpretation of results from GMO experiments (Kurihara, 2001; Mullins and Mullins, 2002; Mullins et al., 2006). The increased use of computer simulations could be helpful, as the elucidation of the paradoxical results of gene titration experiments with the ACE gene have proven (Smithies, 2005). Therefore, Moore suggested systems biology for the detection, characterisation and interpretation of gene–gene interactions (Moore et al., 2006).

With regard to GMO studies, standardisation of the animal experiments is recommended, as the handling and housing of laboratory animals has been shown to have considerable influence on biological variables (Champy et al., 2004). Further, some researchers argue for a stronger focus on clinical research, as cellular and animal models cannot sufficiently model the human disease (e.g. Lindsay, 2005). Reference is made to the importance of heterozygotes of knock-out animals. These animals show the effects of quantitative expression of the gene in question. When genetic variation in the gene leads to hypertension both in heterozygous and homozygous animals, this can indicate a role of respective polymorphisms in humans (Takahashi and Smithies, 2004).

The elucidation of the effects of natural antihypertensives on the molecular level could also lead to improvements in the drug development process. One review investigated the antihypertensive effects of red wine. The activation of eNOS appears to play a decisive role (Leighton et al., 2006). Further, the effects of natural antihypertensive substances, such as linseed (Omoni and Aluko, 2006) or ginseng root (Liu et al., 2006), as well as acupuncture (Kim et al., 2006), have been investigated in animal experiments on the molecular level. Liu explored the antihypertensive effects of resveratrol, a phytoestrogen from grapes. In this case, the inhibition of ET-1 expression appears to play a role (Liu et al., 2003).

## **4 Discussion**

Transgenic and knock-out mouse models for hypertension have existed since 1990 (Kurihara, 2001). Until 2001, it was believed that the results from animal experiments could be extrapolated relatively quickly to humans, despite species-specific differences. However, five years later, researchers became more careful with their interpretations. According to the newer publications, it is not always clear whether blood



pressure changes in GMO are a direct or indirect consequence of the genetic manipulation.

The extrapolation from animal experiments to humans must be considered in the meantime with much more caution. Today, it can no longer be assumed that every gene that plays a role in hypertension in an animal is also relevant in humans. As a multifactorial disease, hypertension can be the result of many small increments of blood pressure resulting from various genetic and environmental changes. Genetic predisposition and physiological adaptation to the environment can contribute equally. A simple, monogenic cause is clearly excluded. It appears that many small genetic modifications make contributions that are not problematic in isolation, but lead to increased blood pressure when combined (Mullins et al., 2006). Such multifactorial causation becomes clear in clinical studies with known antihypertensive drugs, such as ACE inhibitors, beta blockers and calcium channel blockers. Beside decreased blood pressure, other effects are observed that cannot be attributed solely to drug effects (Sever et al., 2006).

In the opinion of many researchers, a targeted genetic modification is a starting point for the identification of interactions that lead to hypertension, although the resulting phenotypes of GMO are still complicated. According to Mullins and colleagues (2006), the homeostasis of the blood pressure is under polygenic control, so that the body can overcome the effects of every mutation by complementary mechanisms that normalise the blood pressure. The genetic complexity of each individual provides the ability to adapt to such changes, or to environmental alterations. Why should this be different in GMO?

From the perspective of the present evidence, some other questions remain unanswered:

To what degree do animal and human physiologies correlate with respect to blood pressure and its regulation? What relevance do the knock-out or transgenic phenotypes have? Do complementary regulatory mechanisms prevent the identification of the true function of knock-out or overexpressed genes? To what degree can mouse genetics aid in the development of new human therapies and the search for new target molecules?

## 5 Conclusions

The intention of the transgenic and knock-out approach in hypertension has always been to make an important contribution to cardiovascular research and thus provide indications for new therapeutic approaches. This is apparent from the motivations of the different research approaches stated in most of the publications examined.

However, it has become clear in the course of this investigation that the publications dealing with basic research have not yet provided any indications for possible applications of the results and have provided no basis for human diagnostic or therapeutic application. The abstracts of the publications dealing with applied research contained some indications of possible applications of the results for therapeutic purposes, but no concrete therapeutic procedures. The potential human benefit in

such cases remains speculative. None of the publications examined contained indications of direct applications of results gained by using GMO, whether in humans or animals. These results were expected given the complexity of hypertension, as we have hardly begun to understand its genetic causes and have no means of controlling the environmental influences that interact with such genetic factors.

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