Cardiovascular diseases identification of genomic markers

Potential interest, limitations

➢ *Degenerative cardiovascular diseases*

- Complexity of anatomical and clinical phenotypes
  (arterial remodeling, obstruction, elevation of BP, cardiac ischemia, preconditioning, arrythmia)
- Well documented hereditary influence but superimposition of environnemental, life style related factors (diet, smoking)

➢ *Genomic variations*

- Robust, reproducible, easily accessible, diagnostic value, may lead to identification of causal factors
- Medical interest relies on clinical association (**studies design and power, quality of clinical phenotyping are major parameters for accuracy)**

Validation of the concept : A few monogenic forms of these diseases have been identified

Inserm «Vascular and Renal Physiology and Pharmacology»
EVGN, FP6

MONOGENIC FORMS OF COMPLEX ARTERIAL DISEASES

- *Familial hypercholesterolemia, mutation in LDL receptors*
  Lipid disorders, Atherosclerosis, Early onset myocardial
  Goldstein and Brown NEJM 1976

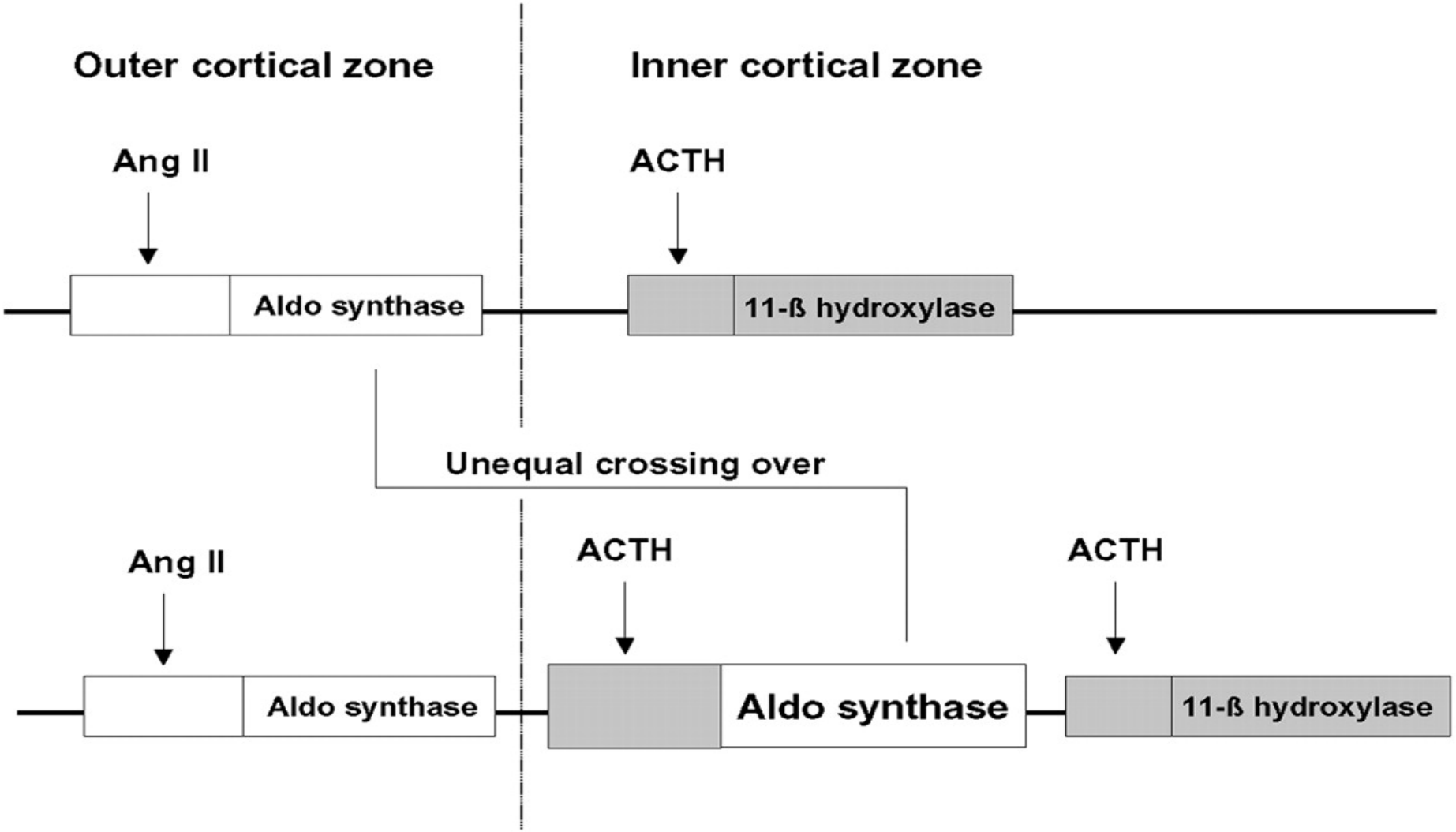
- *Mutation-fusion in 11 betahydroxylase/aldosterone synthase gene*
  Hypokalemia, Glucocorticoid-remediable hyperaldosteronism, High blood pressure
  Lifton et al Nature 1992

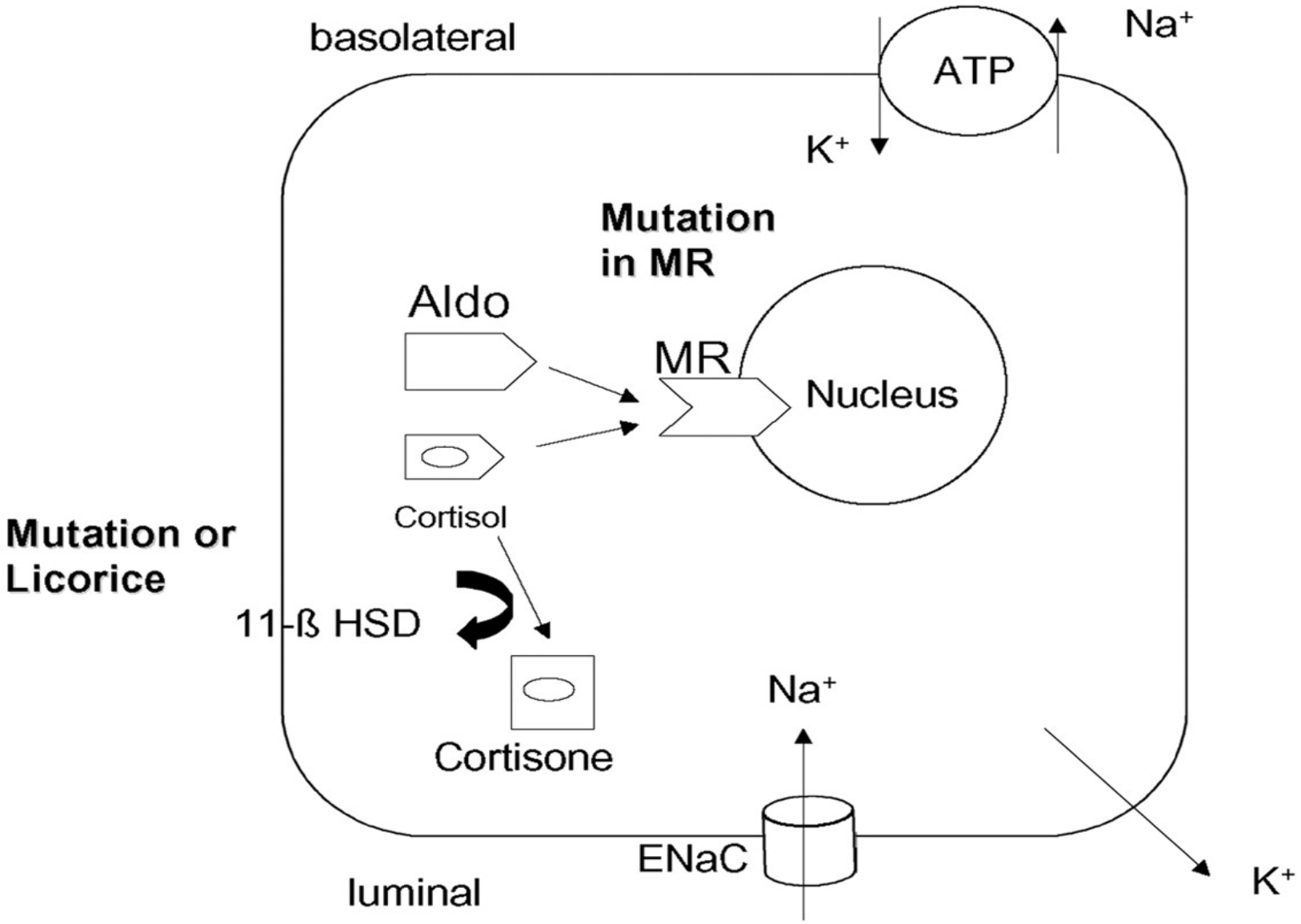
- *Mutations in WNK kininases*
  High blood pressure, Hyperkalemia
  Wilson et al Science 2001

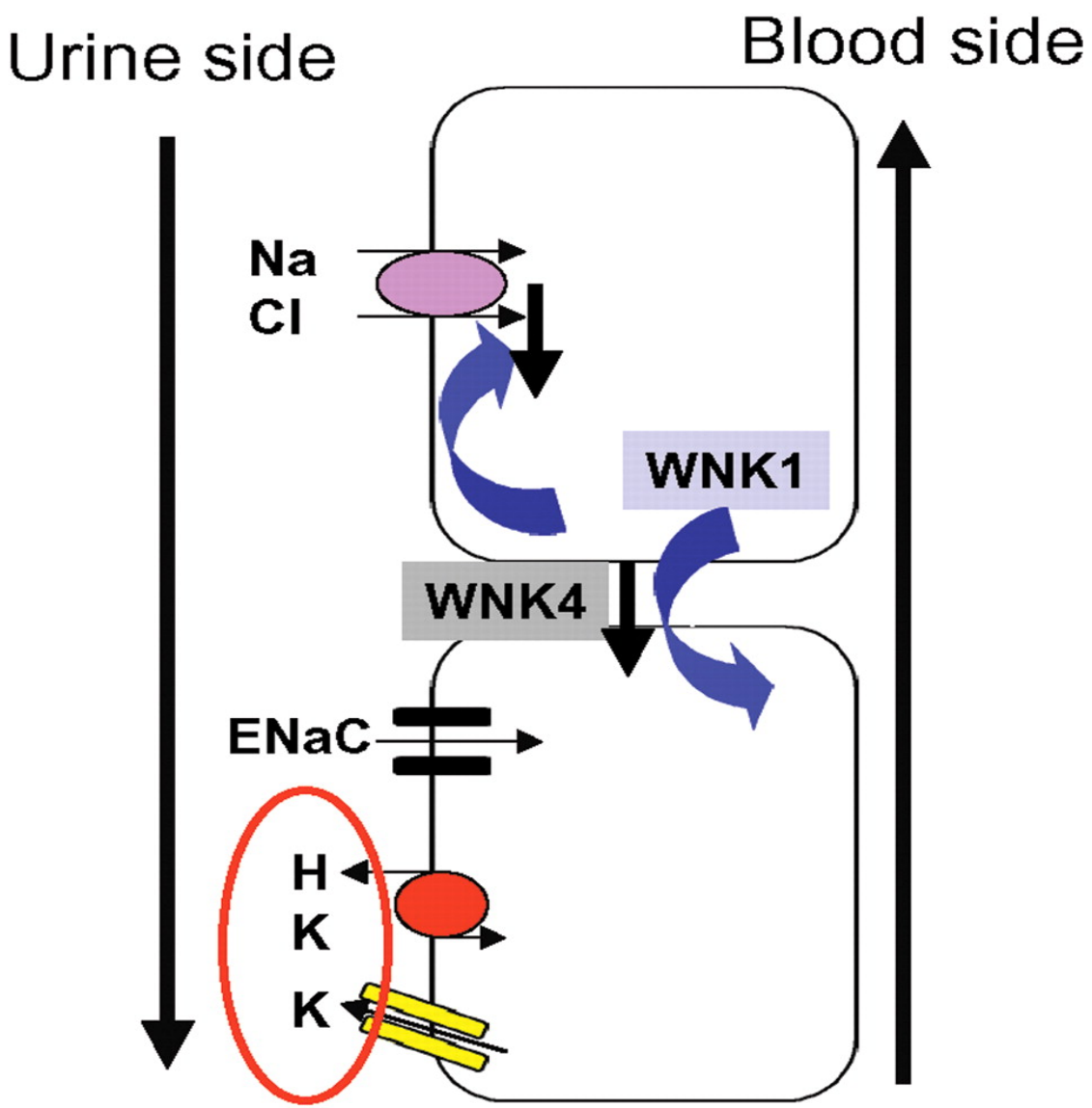
- *Mutation in mitochondrial tRNA*
  High blood pressure, Hypomagnesia, Pure maternal transmission
  Wilson et al Science 2004

Interest: genomic marker gives diagnosis, causality and pathophysiology
Limitation: rare diseases with peculiar, hereditary phenotype, no detectable impact on high blood pressure or MI prevalence in population

Chimeric gene with 11-ß OH promoter and coding region of Aldo synthase




basolateral
ATP
Na+
K+
Mutation
in MR
Aldo
MR
Nucleus
Cortisol
Mutation or
Licorice
11-ß HSD
Cortisone
Na+
ENaC
luminal
K+


Urine side
Blood side
Na
Cl
WNK1
WNK4
ENaC
H
K
K

IDENTIFICATION OF GENOMIC MARKERS FOR CARDIOVASCULAR DISEASES SUSCEPTIBILITY

POPULATION STUDIES

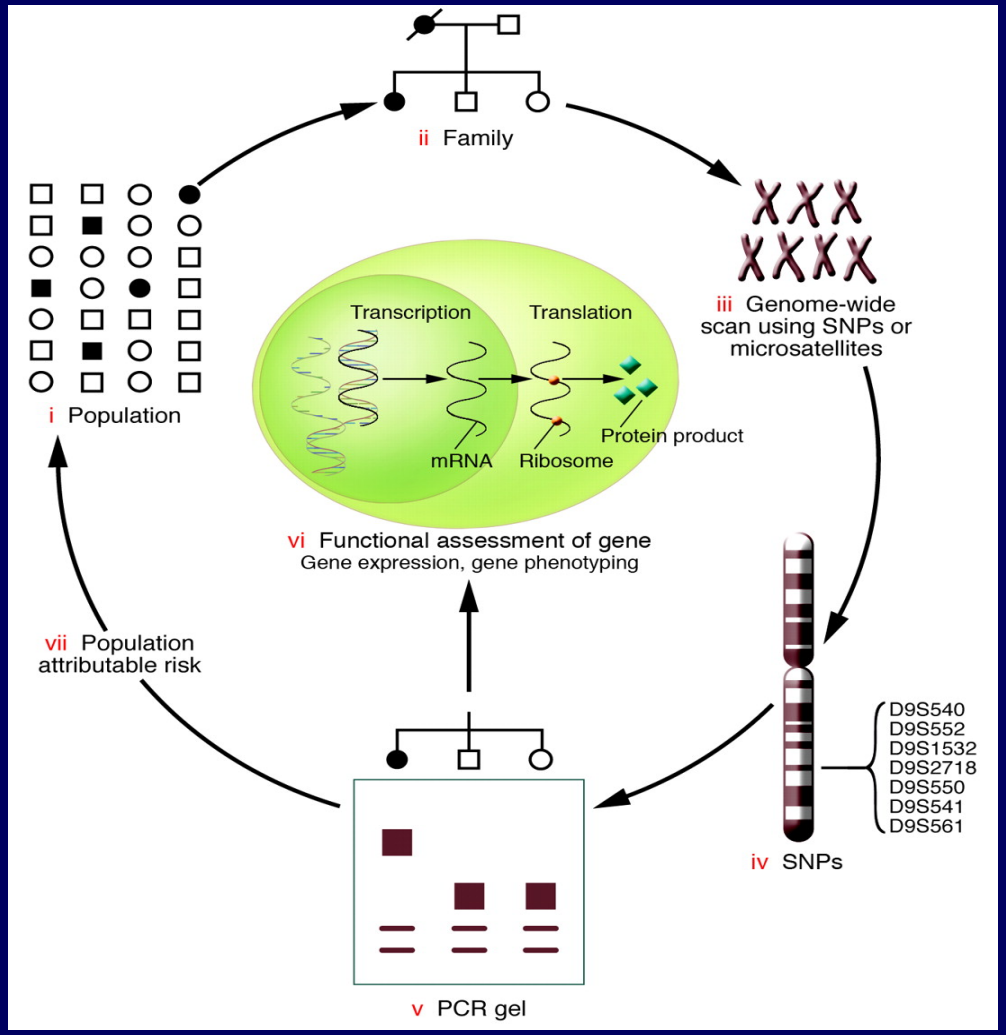
- Genome wide scan
- Candidate gene approach

ANIMAL STUDIES

- Development of strains with spontaneous, hereditary transmissible diseases
- Developement of consomic and congenic rat strains.

Progression of gene mapping in genetic epidemiological studies

Genome wide scan approach



Mayeux, R. J. Clin. Invest. 2005;115:1404-1407

GENOME WIDE SCANS FOR CARDIOVASCULAR DISEASES

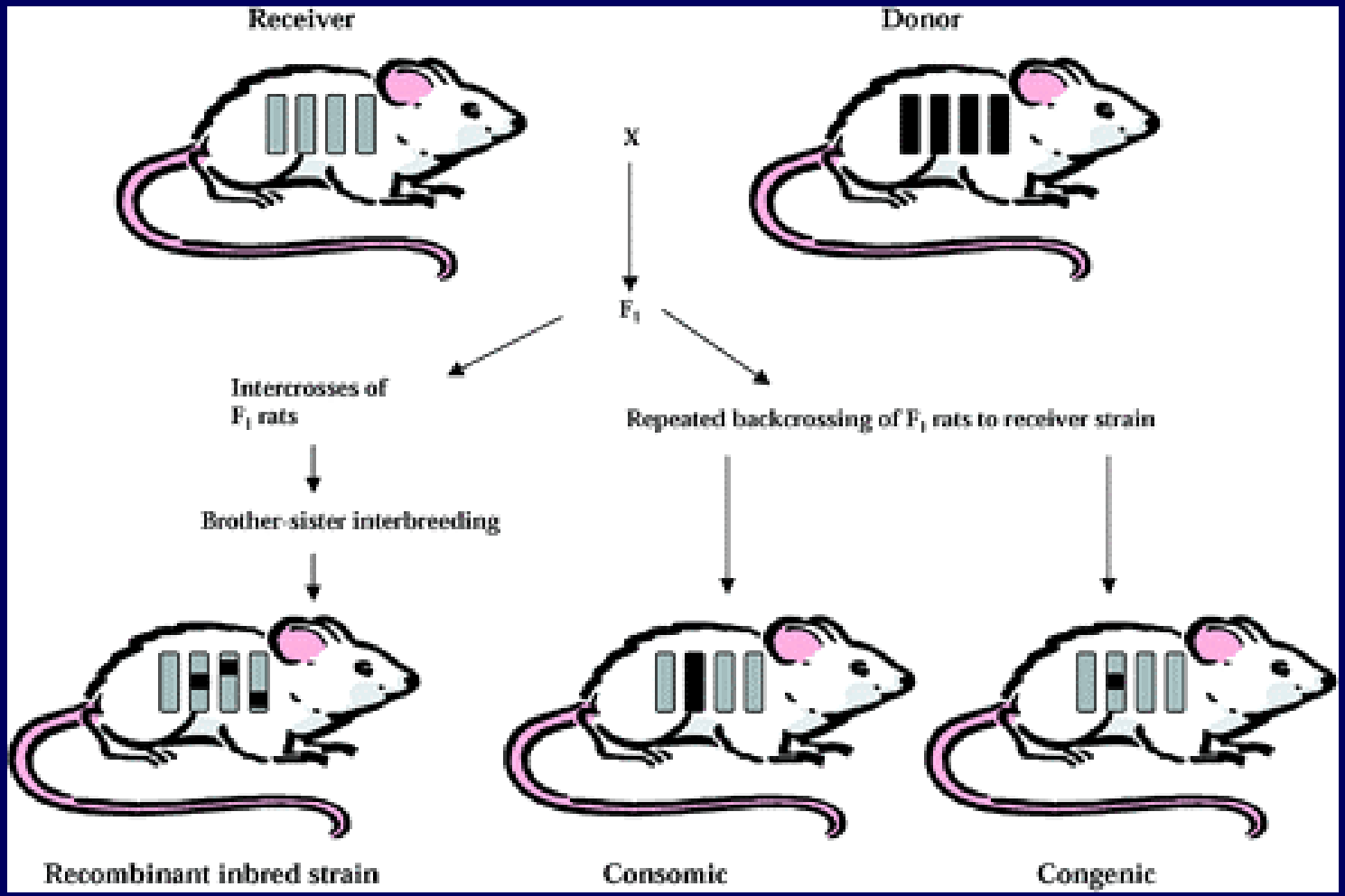
- **High blood pressure and related traits** (pulse pressure, arterial stifness)
  > 30 Studies
  * Many Chromosomes, 1, 2, 11, 16, 21, Y
  * *2 genes identified in monogenic* forms (WNK1, WNK4)

- **Myocardial infarction, early onset coronary artery disease**
  Hanger et al (GENECARD study) Am. J. Hum. Genet. Chromosome 3q, 5q
  Harrap et al ATVB 2003, Chromosome 2
  Franck et al Human Mol Genet 2001, Indomauritians subjects, Chromosome 16

- **Potentially lethal arrythmias**
  Long QT: Newton-Chen et al. Heart and Rythm 2005 Framingham cohort, Chromosome 3
  Neonatal atrial fibrillation: Oberti et al Circulation 2004, Chromosome 5p13

Impact unknown until gene identified

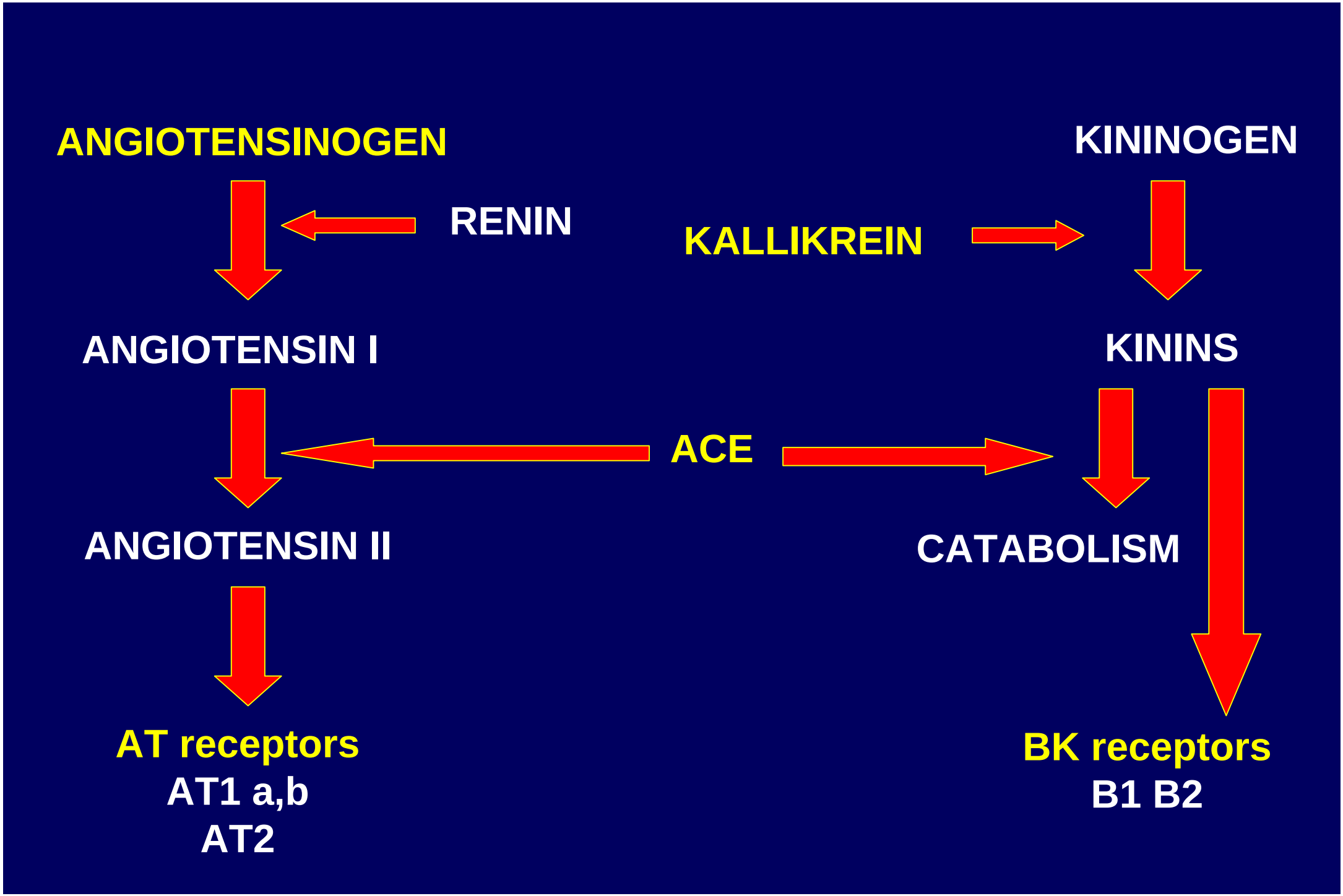
DEVELOPMENT OF CONSOMIC AND CONGENIC ANIMALS



CANDIDATE GENE APPROACH

- **Plausible pathogenic hypothesis (numerous, including vasoactive peptides, sympathetic nervous system, clotting factors, inflammatory mediators, redox factors, homocystein).**

- **Informative genomic polymorphism (linked to a protein product phenotype) and/or multiple polymorphisms of the tested gene.**


ANGIOTENSINOGEN
RENIN
KALLIKREIN
KININOGEN
ANGIOTENSIN I
KININS
ACE
ANGIOTENSIN II
CATABOLISM
AT receptors
AT1 a,b
AT2
BK receptors
B1 B2

GENETIC DETERMINISM OF PLASMA ACE LEVELS

CAMBIEN F, ALHENC-GELAS F, HERBETH B, ANDRE JC, RAKOTOVAO R, GONZALEZ MF, ALLEGRINI J, BLOCH M.
Familial ressemblance of plasma ACE levels. The Nancy Study.
Am. J. Human Genet. 43:774-788, 1988.

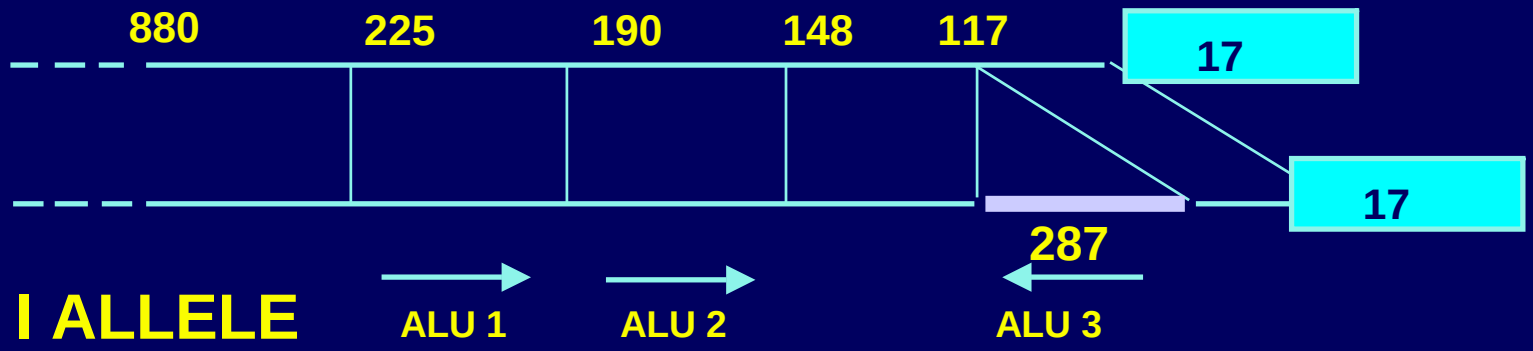
84 nuclear families, 167 children

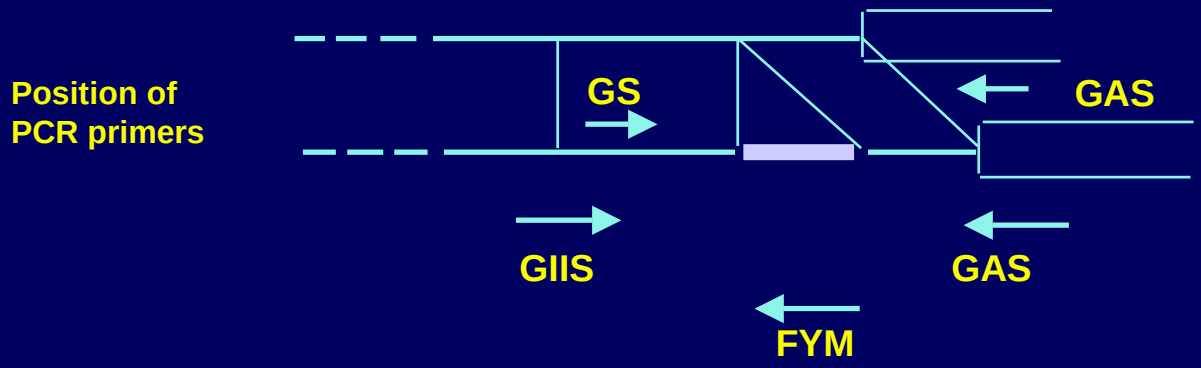
→ Intra familial transmission of plasma ACE levels

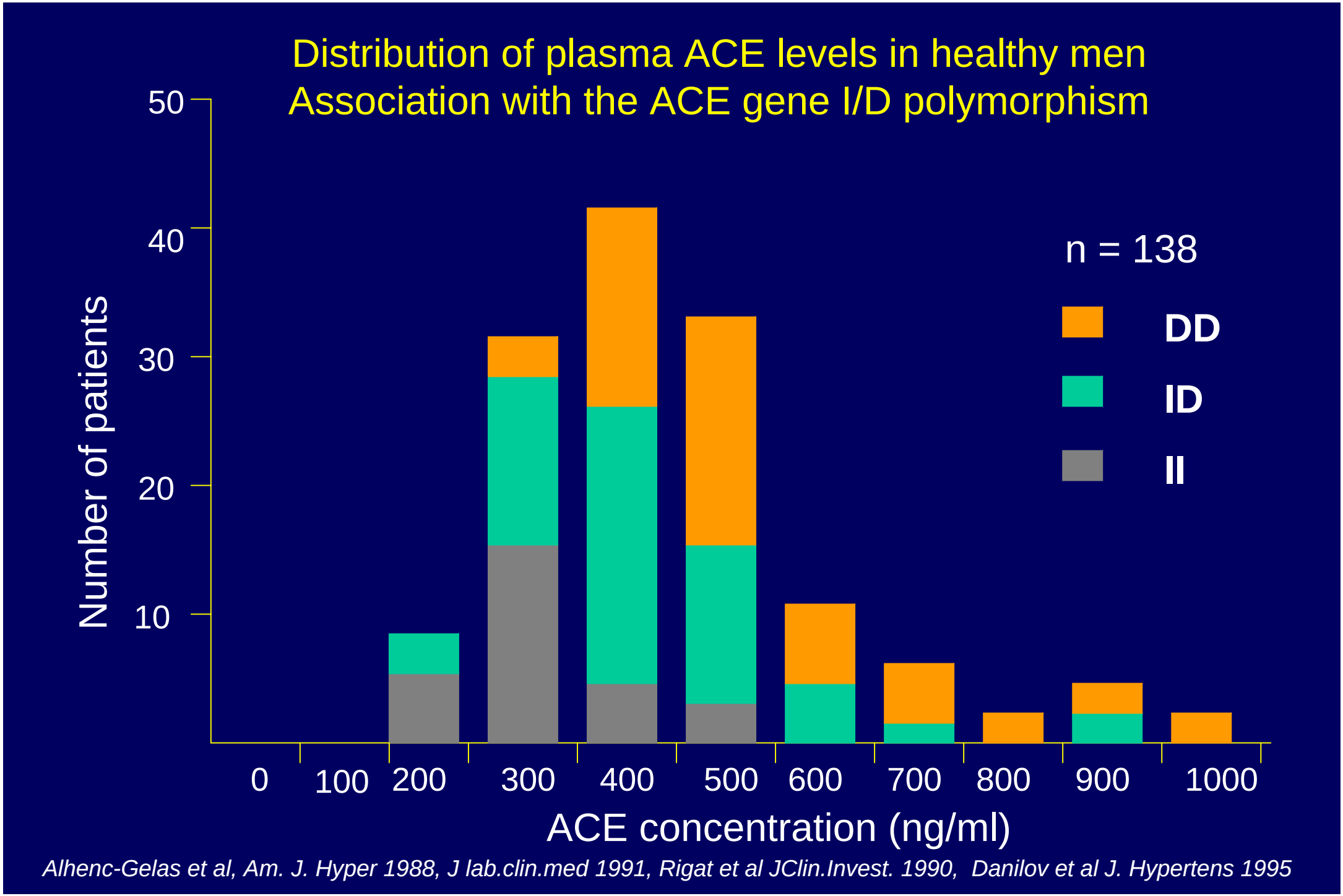
→ Major gene effect

INTRON 16 OF HUMAN ACE GENE
The polymorphic Alu sequence

DALLELE







Alhenc-Gelas et al, Am. J. Hyper 1988, J lab.clin.med 1991, Rigat et al JClin.Invest. 1990, Danilov et al J. Hypertens 1995

GENETIC POLYMORPHISM OF ACE LEVEL AND CARDIOVASCULAR AND RENAL DISEASES

- *Familial ressemblance of plasma ACE levels*

- *Molecular cloning, ACE gene ID polymorphism, Association with ACE levels*

- *Coronary insufficiency, myocardial infarction*
  - Cambien et al Nature 1992, Circulation 1994
  > 50 studies, consensus not reached

- *Vascular and renal complications of diabetes, diabetic nephropathy*
  -Marre et al Diabetes 1994, J Clin Invest I 1997
  > 50 studies, reproducibility demonstrated, causality established
  Cohort studies (Parving et al BMJ 1996, Hadjadj et al JASN 2001, Boright et al DCCT-DCI, Diabetes 2005)
  Animal models ⟶ Causative link, Molecular mechanisms

DIABETIC NEPHROPATHY
(Type I Diabetes)

- Major complication of chronic hyperglycemia.

-Elevated microalbuminuria (early stage of DN) is associated with a 3 to 5 fold increase in major cardiovascular events (myocardial infarction). DN is a also a cardiovascular disease

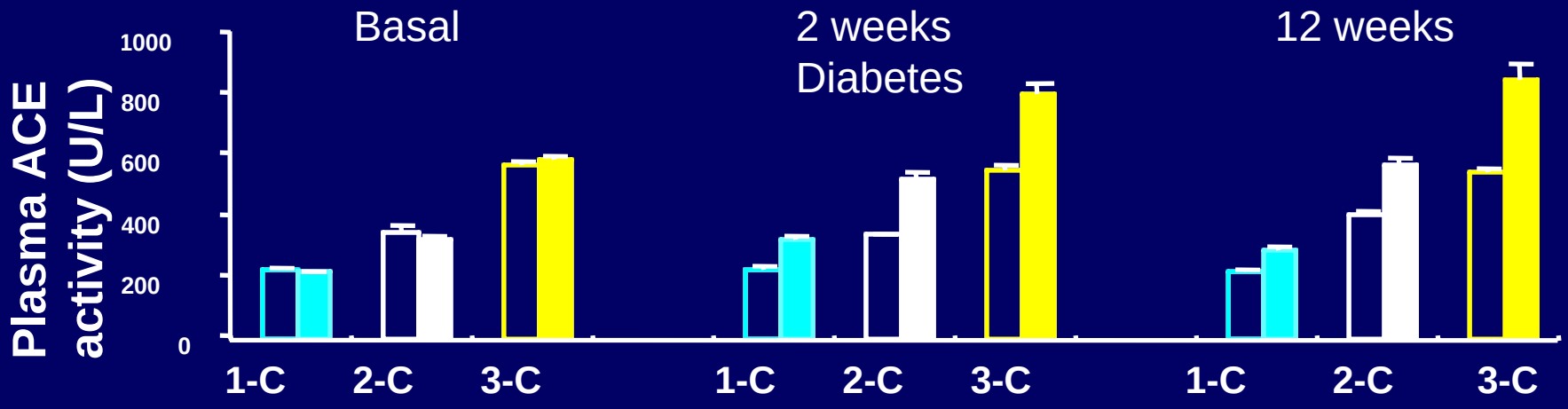
- Risk is genetically determined.

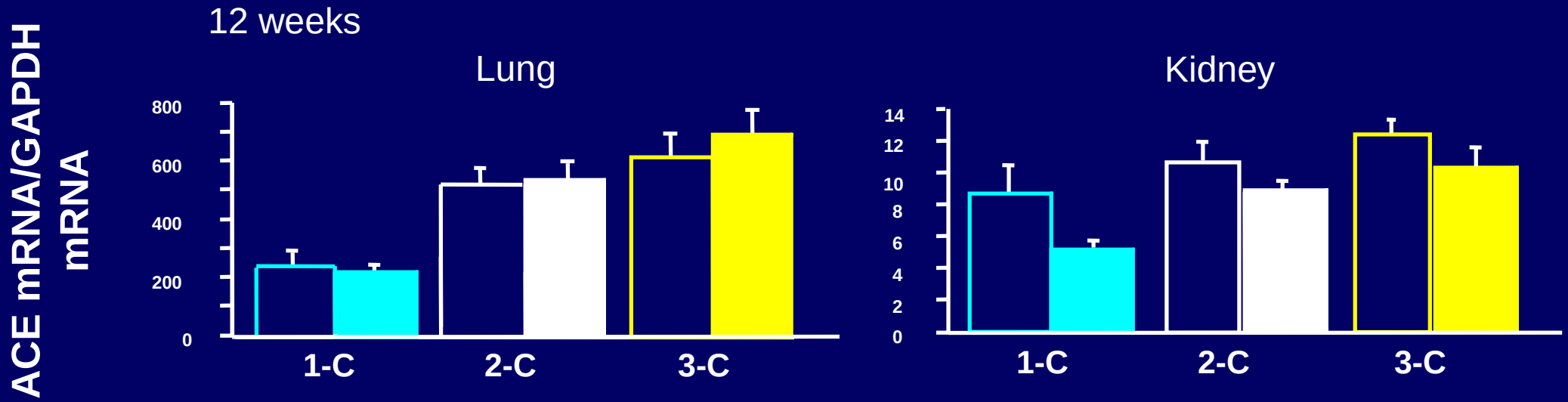
Follow-up study. Determinants of DN. Cox proportional hazard model for the 310 patients

Hadjadj et al JASN 2001	Adjusted hazard ratio	95% CI	p value
Sex (Male compared to Female)	1.56	0.83-2.91	0.1658
Mean HbA1c during follow-up (each increase of 1%)	1.36	1.08-1.70	0.0087
Baseline SBP (compared to less than 115 mmHg)	/	/	0.0646
115 to 124	1.74	0.62-4.89	0.2953
125 to 134	1.54	0.50-4.70	0.4521
135 or more	3.51	1.21-10.18	0.0211
Diabetes duration (compared to less than 10 years)	/	/	0.3682
10 to 19	1.48	0.72-3.03	0.2880
20 or more	0.92	0.41-2.04	0.8378
Baseline nephropathy stage (compared to stage 1)	/	/	0.4347
stage 2	0.59	0.23-1.92	0.3257
stage 3	1.47	0.56-3.85	0.4370
stage 4	1.92	0.53-6.93	0.3218
ACE polymorphism (ID or DD compared to II genotype)	5.00	1.51-16.57	0.0086

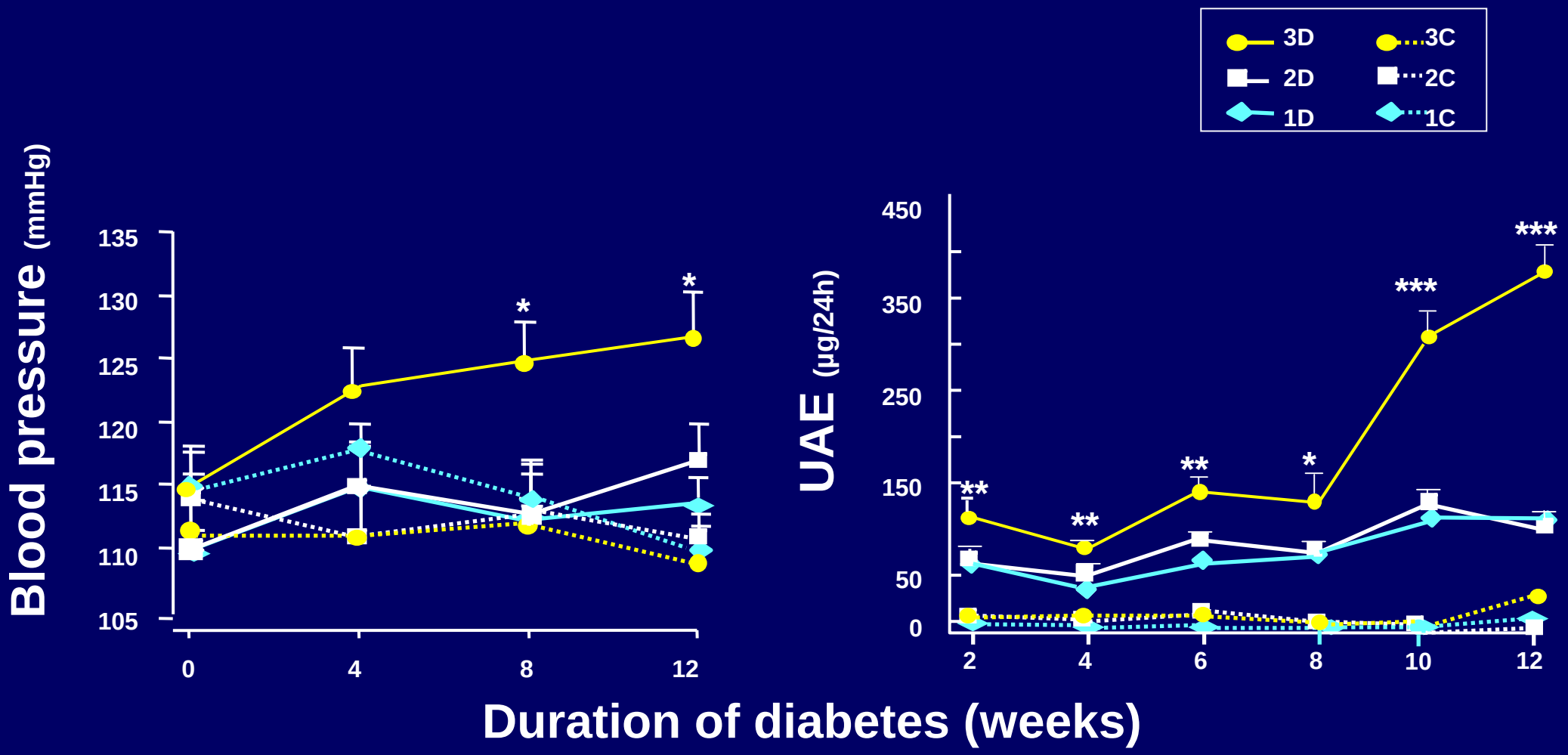
ACE GENE TITRATION IN THE MOUSE

(Inactivation or Duplication, 1 to 3 copies)



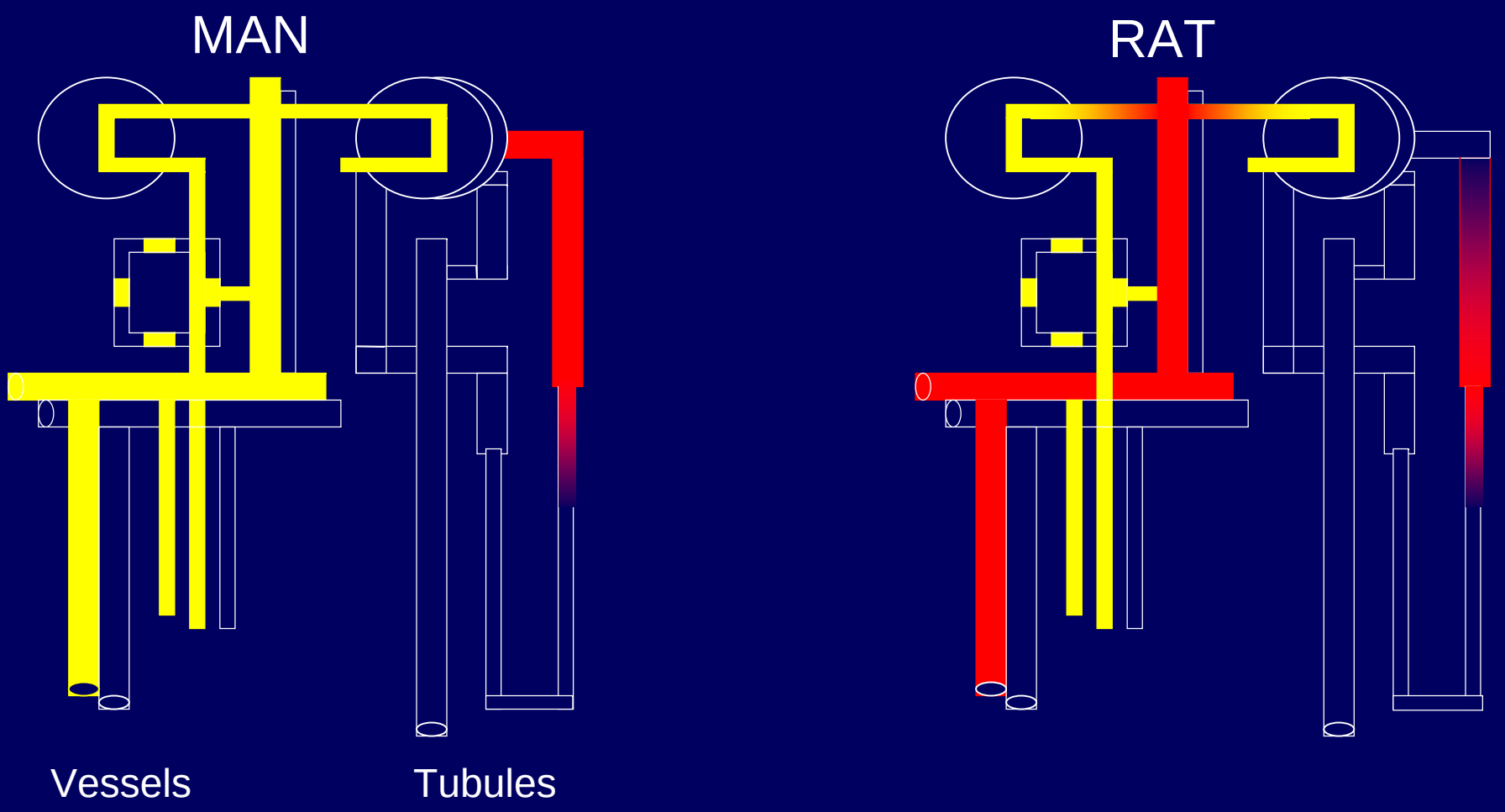


EFFECT OF ACE GENE COPY NUMBER IN CONTROL AND DIABETIC MICE



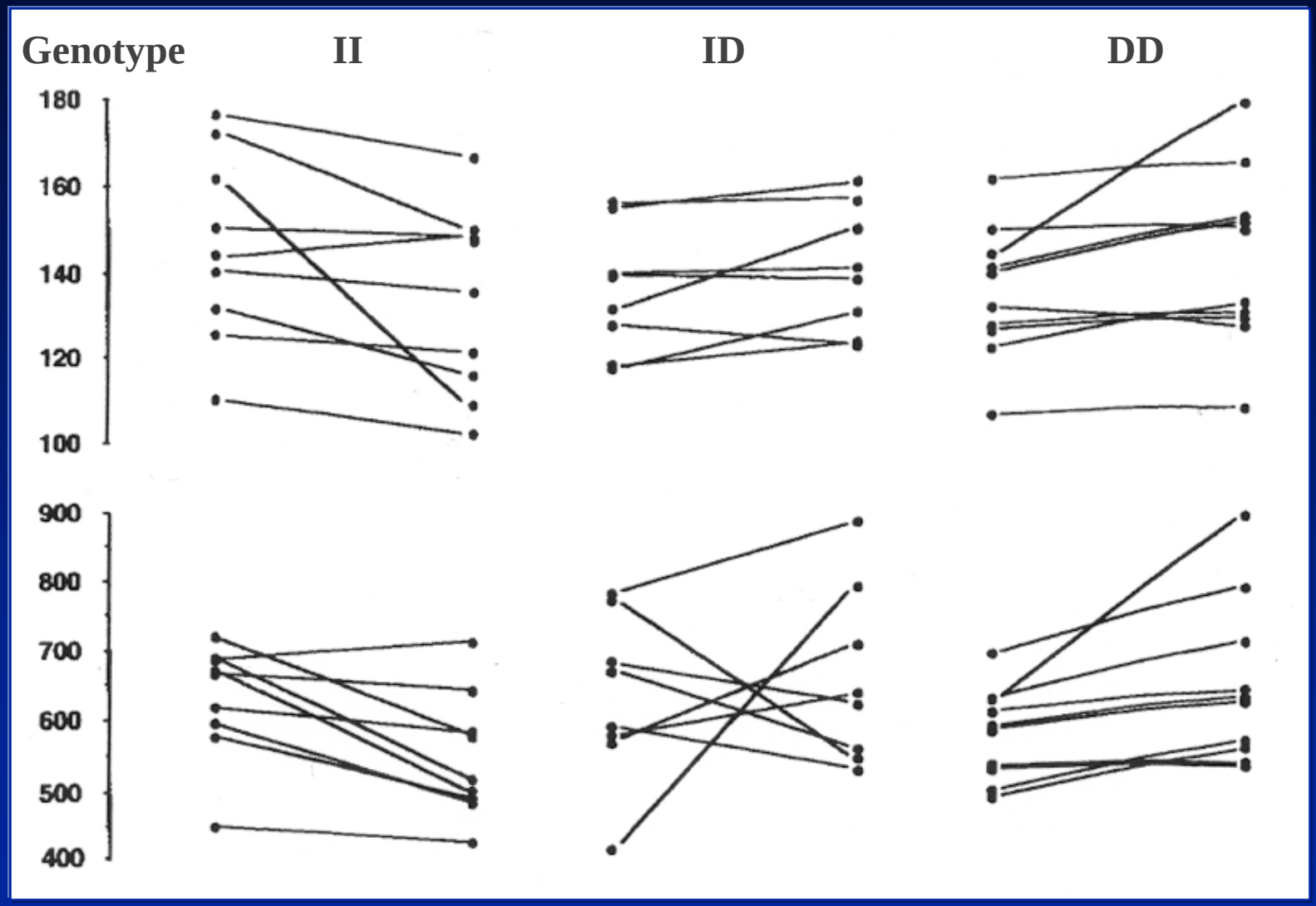
Huang et al PNAS 2001

LACK OF ENDOTHELIAL ACE IN THE HUMAN KIDNEY



Franke et al KI, 1999

Renal response to glucose infusion in normoalbuminuric IDDM patients according to the ACE gene polymorphism



Effect of D allele p<0.05

Marre et al Hypertension 1999

ESTABLISHMENT OF A GENOMIC VARIATION AS A RISK MARKER FOR CARDIOVASCULAR DISEASE FROM A RISK MARKER TO A RISK FACTOR

- Initial observation: association studies
  - Case control
  - Cohort
- Replication in other cohorts

  Review of individual studies, meta-analyses, consensus
- Establishment of causality
  - Genetic animal studies
- Mechanistic insight
  - Animal and clinical studies

ACE and Diabetic vascular complications :10 years

Potential thérapeutic applications

→ *Identification of gene product as a potential drug target*

→ *Interaction with already identified treatments. Refinement of therapeutic strategies.*

INTERACTION BETWEEN ACE GENE POLYMORPHISM AND DISEASE OUTCOME UNDER TREATMENT (ACE inhibitors, other treatments)

> 30 Studies, analysis of clinical intervention trials according to genotype.

- Diabetes Complications

Penno et al Diabetes 1998
Pera et al Kid Int 2000
Jacobsen et al J. Am. Soc. Nephr. 2003
Diabhycargene study

- Congestive Heart Failure

Mac Namara et al Circulation 2001, J. Am. Col. Cardiol. 2005
Cicoria et al Am. J. Med. (2201, 2004) (Spirolactone)

Need of dedicated studies, with appropriate design and power.
High potential medical interest. Support ?