Common and rare variants influencing blood pressure

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Use of genetic data to inform cardiovascular care, the example of blood pressure
Large genomics studies: examples

Traditional population-based and clinical studies. Sample sizes in general <20k participants.

- **THE PRECISION MEDICINE INITIATIVE**
  - Budget: 215mio USD
  - 500’000 participants

- **KAISER insurance**
  - Example of EHR-based study
  - Rare disease

**Additional Resources**
- www.whitehouse.gov/precision-medicine
- www.dor.kaiser.org/external/DORExternal
Blood pressure genomics as an example cardiovascular complex phenotype

Types of BP genetics.

Results from genome-wide association studies (GWAS) and how we can learn from them.

A self-experiment using 23andMe.
Prevalence of HTN in Switzerland

- **CoLaus** Study: 6,182 participants from 2003-2006
  - 35-75y
  - 52% female
- HTN in 36%
- **SWISSHYPE** (2009 survey on 1,376 hypertensives)
  - 65+-12y
  - 54% male

Prevalence (%) of awareness, treatment and control in men and women aged 35–75 years with high blood pressure (defined as blood pressure ≥ 140/90 mmHg or antihypertensive medication) from Lausanne, Switzerland.

Population effect of BP reduction

Analysis of 5 major observational studies:

- **SBP by 5 mmHg:**
  - Stroke mortality by 14%
  - CHD mortality by 9%
  - All-cause mortality by 7%

Lewington et al. Lancet 2002; 360:1903
Causes for the distribution of quantitative traits

\[ X \sim N(\mu, \sigma^2) \]

environment \rightarrow X \sim N(\mu, \sigma^2) \rightarrow genetics

<table>
<thead>
<tr>
<th>Trait</th>
<th>heritability</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>42%^1</td>
</tr>
<tr>
<td>DBP</td>
<td>39%^1</td>
</tr>
<tr>
<td>T2D</td>
<td>26%^2</td>
</tr>
<tr>
<td>TC</td>
<td>64%^3</td>
</tr>
<tr>
<td>LDL</td>
<td>66%^3</td>
</tr>
<tr>
<td>HDL</td>
<td>58%^3</td>
</tr>
<tr>
<td>TG</td>
<td>42%^3</td>
</tr>
<tr>
<td>CAD</td>
<td>56%^4</td>
</tr>
</tbody>
</table>

Kurt Stern, Benetton, controllinghighbp.com
Human genome and genetic variation

• **SNPs**
  - most frequent type of variation (38m SNPs in 1000G)
  - variant load per individual: ~4m (1000G)

  ![SNP population level](image)

  *Population level:*
  - common SNPs: >5% MAF
  - low frequency SNPs: 0.5-5% MAF
  - rare SNPs <0.5% MAF

• **others**
  - INDELS and CNV (1.4m INDELS in 1000G).
  - Epigenetic modifications.


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The older a variant, the more frequent it generally is.

-> Common trait – common variant hypothesis.

Aravinda Chakravarti, Nature Genetics 1999;21:56
Allele frequency and effect size

- Rare alleles causing Mendelian disease
- Low-frequency variants with intermediate effect
- Common variants implicated in common disease by GWA
- Few examples of high-effect common variants influencing common disease

Manolio et al, Nature 2009; 461, 747-753
Blood pressure (BP): monogenic and quantitative trait

Families with “Lifton genes” (e.g. familial hyperkalemic hypertension)

Classic genetic quantitative trait

Victor McKusick, Circulation 1960;5:857; Mayan H et al. JCEM 2009;94:3010-3016
# Monogenic hypertensive syndromes: “Lifton genes”

<table>
<thead>
<tr>
<th>Gene(s)</th>
<th>Chr</th>
<th>Disease name</th>
<th>Key features of clinical syndrome</th>
<th>Estimated frequency; occurrence in the general population</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP11B1</strong> (11-beta hydroxylase gene)</td>
<td>8q</td>
<td>(MIM 202010) Congenital adrenal hyperplasia, due to 11-beta-hydroxylase deficiency</td>
<td>hypertension, hypokalemia, virilization (variable); 2/3 of patients have severe, “classic form” with HTN in first years of life, otherwise hypertension is usually mild to moderate in intensity; accounts for 5-8% of all CAH cases</td>
<td>~1/100,000 births ~1/5-7,000 in Jewish families of North African origin (Morocco, Tunisia)</td>
</tr>
<tr>
<td><strong>CYP11B2</strong> (aldosterone synthase gene)</td>
<td>8p</td>
<td>(MIM 103900) Glucocorticoid remediable aldosteronism = familial hyperaldosteronism type I = glucocorticoid suppressible hyperaldosteronism</td>
<td>hypertension, low plasma renin, increased aldosterone, response to dexamethasone; high genetic heterogeneity and potassium level often normal; high prevalence of intracranial aneurysms</td>
<td>rare defect</td>
</tr>
<tr>
<td><strong>WNK1, WNK4</strong> (lysine-deficient protein kinase 1 &amp; 4 genes)</td>
<td>12p</td>
<td>Pseudohypoaldosteronism type 2 (PHA2) = Gordon syndrome WNK1: PHA2C (MIM 614492) WNK4: PHA2B (MIM 614491) KLHL3: PHA2D (MIM 614495) CUL3: PHA2E (MIM 614496)</td>
<td>hypertension, hypokalemia, response to thiazides</td>
<td>rare defect</td>
</tr>
<tr>
<td><strong>SCNN1B, SCNN1G</strong> (amilorid-sensitive sodium channel, beta &amp; gamma subunit gene encoding two subunits of the ENaC sodium channel)</td>
<td>16p</td>
<td>(MIM 177200) Liddle syndrome = pseudoaldosteronism</td>
<td>hypertension, hypokalemia, metabolic alkalosis, low plasma renin, low aldosterone, respond to amiloride</td>
<td>rare defect</td>
</tr>
<tr>
<td><strong>CYP17A1</strong> (steroid 17-hydroxylase / 17,20 lyase gene)</td>
<td>10q</td>
<td>(MIM 202110) Congenital adrenal hyperplasia, due to 17-alpha-hydroxylase deficiency = CAH type V</td>
<td>hypertension, hypokalemia, hypogonadism / androgen deficiency</td>
<td>very rare defect</td>
</tr>
<tr>
<td><strong>HSD11B2</strong> (11-beta-hydroxy steroid dehydrogenase 2 gene)</td>
<td>16q</td>
<td>(MIM 218030) Cortisol 11-beta-ketoreductase deficiency = syndrome of apparent mineralocorticoid excess</td>
<td>hypertension, hypokalemia, low plasma renin, responsiveness to spironolactone; severe hypertension</td>
<td>very rare defect</td>
</tr>
<tr>
<td><strong>NR3C2</strong> (mineralocorticoid receptor gene)</td>
<td>4q</td>
<td>(MIM 605115) Early onset autosomal dominant hypertension with exacerbation in pregnancy</td>
<td>hypertension, severe hypertension in pregnancy</td>
<td>one large pedigree reported</td>
</tr>
<tr>
<td><strong>KCNJ5</strong> (potassium inwardly-rectifying channel gene, subfamily J, member 5)</td>
<td>11q</td>
<td>(MIM 613677) Familial hyperaldosteronism type III</td>
<td>hypertension, hypokalemia, high aldosterone, high 18-oxocortisol and 18-hydroxycortisol</td>
<td>one pedigree reported</td>
</tr>
</tbody>
</table>

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BP in the general population

• No significant contribution by the monogenic BP variants (they are all very rare).

• No significant contribution by the genes in known BP pathways (these have been sequenced in large numbers).
Technological development of large-scale genotyping in 2000-2005

La technologie « microarray » permet de génotyper des millions de SNPs en une expérience.

Le génotypage / séquencage devient plus accessible.

Le coût pour le génotypage / séquencage baisse plus rapidement que la loi de Moore.


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Complex trait genomics 2005-2016

GWAS findings: end 2005

End 2016
Genome-wide association studies, cont.

Association

- SNP
- genotype XY: 5 (of 6) 1 (of 6)
- $\chi^2 = 5.3$
- $p = 0.03$
- No. of markers usually utilized: $\sim10^6$

Genome-wide association studies: Multiple testing adjustment

**No. of markers**
usually \( \sim 10^6 \)
Significance threshold:

\[
P = \frac{0.05}{1,000,000} = 5 \times 10^{-8}
\]
(Bonferroni)
State of HTN/BP GWAS in 2007

WTCCC GWAS 2007: cardiovascular traits or risk factors: 2,000 cases / 3,000 ctr.

Power of BP GWA studies

The effect size observed for BP per variant is \( \sim 1 \text{mmHg} = \sim 0.05 \text{ SD for SBP} \)

EHJ 2013 Apr;34(13):951-61
Five observations from BP genomics studies.

1) The GWAS BP loci are largely in regions previously unknown to be relevant for BP

Example from 2009 GWAS:

Gene name(s) near each locus

chr1: moloney leukemia virus 10 homolog; ST7L
chr1: MTHFR reductase; natriuretic peptide precursor B
chr3: solute carrier family 4 (sodium bicarb. transporter)
chr3: MDS1 and EVI1 complex locus
chr3: unc-51-like kinase 4
chr4: solute carrier family 39 (zinc transporter)
chr4: soluble guanylate cyclase 1
chr4: fibroblast growth factor 5 precursor
chr5: natriuretic peptide receptor C
chr5: early B-cell factor
chr6: hemochromatosis protein
chr6: HLA-B associated transcript (BAT2)
chr10: voltage-dependent calcium channel
chr10: phospholipase C epsilon 1
chr10: unknown
chr10: cytochrome 17A1; 5'-nucleotidase
chr11: adrenomedullin 2 precursor
chr11: rho-type GTPase-activating protein
chr11: pleckstrin homology domain containing protein
chr12: plasma membrane calcium ATPase 1
chr12: SH2B adaptor protein 3
chr12: T-box 3 protein; T-box 5 protein
chr15: feline sarcoma oncogene
chr15: cytochrome 1A1; unc-51-like kinase 3
chr17: golgi SNAP receptor complex member 2
chr17: zinc finger protein 652
chr20: jagged 1 precursor
chr20: GNAS complex; endothelin 3 isoform 3; C20orf66
Five observations from BP genomics studies.

2) The number of associated variants is large and their frequency is generally common (average MAF ~30%), there are only few examples of uncommon / rare variants.
Five observations from BP genomics studies.

3) Typical effect sizes per risk allele are **small and little of the total trait variance is explained.**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Typical absolute effect size of one SNP</th>
<th>Effect explained (of total phenotype variation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP/DBP</td>
<td>~1/0.5 mmHg</td>
<td>~3-4%</td>
</tr>
<tr>
<td>LDL/HDL/TG</td>
<td>~0.02mmol/L</td>
<td>~10%</td>
</tr>
<tr>
<td>diabetes</td>
<td></td>
<td>~6%</td>
</tr>
</tbody>
</table>

Locke et al. Nature. 2015 Feb;518(7538):197-206
### Five observations from BP genomics studies.

4) The same BP variants appear to act in all ethnicities.

Association with BP in non-white ethnicities using a 29-SNP risk score:

<table>
<thead>
<tr>
<th>Ancestry</th>
<th>N</th>
<th>Systolic BP</th>
<th>Diastolic BP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Effect size</td>
<td>S.E.</td>
</tr>
<tr>
<td>East Asian</td>
<td>29,719</td>
<td>1.06</td>
<td>0.08</td>
</tr>
<tr>
<td>South Asian</td>
<td>23,977</td>
<td>0.55</td>
<td>0.08</td>
</tr>
<tr>
<td>African &amp; AD</td>
<td>19,775</td>
<td>0.41</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*per SD of the 29-SNP risk score

5) Improved phenotype precision seems to help identification of BP loci.

**LTA** in 48,564 European participants: up to 4 measurements averaged within a 15 year time-span, visits were at least one year apart.

**Correlation between LTA and “single visit” phenotype:**

- **SBP** Residual v.s. SBP Visit 1 Residual, in ARIC
- **DBP** Residual v.s. DBP Visit 1 Residual, in ARIC (N=8775)
ICBP LTA analysis

Across all phenotypes 20 loci reach genome-wide significance. Of these 4 are new (chr2:26Mb, chr2:96Mb, chr6:43Mb, chr7:45Mb) and replication was attempted in 40,254 individuals with single visit association results (GBPGen).
What is this information useful for?

• Investigation of cause-effect relationships.
• Identification of new pathways.
• Identification of causal tissues.
Mendelian randomization studies

GENES → BP → CAD

GENE → BP  CAD

XYZ
# Mendelian randomization of BP effects using 66 SNPs

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Variant type</th>
<th>Ancestry</th>
<th>Total n or cases/controls</th>
<th>Total SNPs</th>
<th>Effect (all)</th>
<th>( P(\text{all}) )</th>
<th>( P(\text{int}) ) (all)</th>
<th>( P(p) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart Coronary artery disease</td>
<td>Dichotomous</td>
<td>EUR_SAS</td>
<td>63,746/130,681</td>
<td>61</td>
<td>1.042</td>
<td>( 1.72 \times 10^{-44} )</td>
<td>( 1.75 \times 10^{-26} )</td>
<td>( 4.08 \times 10^{-32} )</td>
</tr>
<tr>
<td>Heart failure</td>
<td>Dichotomous</td>
<td>EUR</td>
<td>2,526/18,400</td>
<td>66</td>
<td>1.021</td>
<td>( 2.77 \times 10^{-2} )</td>
<td>( 1.63 \times 10^{-1} )</td>
<td>( 2.77 \times 10^{-2} )</td>
</tr>
<tr>
<td>LV mass</td>
<td>Continuous</td>
<td>EUR</td>
<td>11,273</td>
<td>66</td>
<td>0.480</td>
<td>( 6.43 \times 10^{-4} )</td>
<td>( 3.58 \times 10^{-1} )</td>
<td>( 6.43 \times 10^{-4} )</td>
</tr>
<tr>
<td>LV wall thickness</td>
<td>Continuous</td>
<td>EUR</td>
<td>11,311</td>
<td>66</td>
<td>0.004</td>
<td>( 4.45 \times 10^{-6} )</td>
<td>( 5.83 \times 10^{-2} )</td>
<td>( 4.45 \times 10^{-6} )</td>
</tr>
<tr>
<td>Kidney CKD</td>
<td>Dichotomous</td>
<td>EUR</td>
<td>6,271/68,098</td>
<td>65</td>
<td>1.010</td>
<td>( 1.37 \times 10^{-1} )</td>
<td>( 1.77 \times 10^{-3} )</td>
<td>( 2.65 \times 10^{-1} )</td>
</tr>
<tr>
<td>eGFR (based on creatinine)</td>
<td>Continuous</td>
<td>EUR</td>
<td>74,354</td>
<td>65</td>
<td>0.000</td>
<td>( 7.07 \times 10^{-1} )</td>
<td>( 3.12 \times 10^{-5} )</td>
<td>( 3.22 \times 10^{-1} )</td>
</tr>
<tr>
<td>eGFR (based on cystatin)</td>
<td>Continuous</td>
<td>EUR</td>
<td>74,354</td>
<td>65</td>
<td>-0.001</td>
<td>( 9.05 \times 10^{-2} )</td>
<td>( 9.28 \times 10^{-6} )</td>
<td>( 4.11 \times 10^{-1} )</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Continuous</td>
<td>EUR</td>
<td>23,812</td>
<td>66</td>
<td>0.000</td>
<td>( 9.42 \times 10^{-1} )</td>
<td>( 6.31 \times 10^{-3} )</td>
<td>( 9.42 \times 10^{-1} )</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>Dichotomous</td>
<td>EUR</td>
<td>2,499/29,081</td>
<td>65</td>
<td>1.011</td>
<td>( 2.10 \times 10^{-1} )</td>
<td>( 4.79 \times 10^{-2} )</td>
<td>( 2.1 \times 10^{-1} )</td>
</tr>
<tr>
<td>Urinary albumin/creatinine ratio</td>
<td>Continuous</td>
<td>EUR</td>
<td>31,580</td>
<td>65</td>
<td>0.009</td>
<td>( 2.52 \times 10^{-3} )</td>
<td>( 3.02 \times 10^{-4} )</td>
<td>( 0.53 \times 10^{-3} )</td>
</tr>
<tr>
<td>Stroke Stroke, all subtypes</td>
<td>Dichotomous</td>
<td>EUR</td>
<td>1,544/18,058</td>
<td>66</td>
<td>1.058</td>
<td>( 6.11 \times 10^{-6} )</td>
<td>( 8.26 \times 10^{-2} )</td>
<td>( 6.11 \times 10^{-6} )</td>
</tr>
<tr>
<td>Stroke, ischemic subtype</td>
<td>Dichotomous</td>
<td>EUR</td>
<td>1,164/18,438</td>
<td>66</td>
<td>1.069</td>
<td>( 3.33 \times 10^{-6} )</td>
<td>( 1.75 \times 10^{-1} )</td>
<td>( 3.33 \times 10^{-6} )</td>
</tr>
<tr>
<td>Stroke, ischemic subtype</td>
<td>Dichotomous</td>
<td>EUR</td>
<td>11,012/40,824</td>
<td>66</td>
<td>1.036</td>
<td>( 1.69 \times 10^{-10} )</td>
<td>( 4.72 \times 10^{-2} )</td>
<td>( 1.69 \times 10^{-10} )</td>
</tr>
<tr>
<td>Vasculature cIMT</td>
<td>Continuous</td>
<td>EUR</td>
<td>27,610</td>
<td>66</td>
<td>0.004</td>
<td>( 4.80 \times 10^{-15} )</td>
<td>( 5.06 \times 10^{-8} )</td>
<td>( 7.32 \times 10^{-10} )</td>
</tr>
<tr>
<td>Eye Mild retinopathy</td>
<td>Dichotomous</td>
<td>EUR</td>
<td>1,122/18,289</td>
<td>66</td>
<td>1.021</td>
<td>( 1.37 \times 10^{-3} )</td>
<td>( 6.01 \times 10^{-1} )</td>
<td>( 1.37 \times 10^{-3} )</td>
</tr>
<tr>
<td>Central retinal artery caliper</td>
<td>Continuous</td>
<td>EUR</td>
<td>18,576</td>
<td>66</td>
<td>-0.343</td>
<td>( 3.29 \times 10^{-14} )</td>
<td>( 2.56 \times 10^{-6} )</td>
<td>( 2.06 \times 10^{-13} )</td>
</tr>
<tr>
<td>Mild retinopathy</td>
<td>Dichotomous</td>
<td>EAS</td>
<td>289/5,419</td>
<td>66</td>
<td>1.033</td>
<td>( 2.55 \times 10^{-1} )</td>
<td>( 2.42 \times 10^{-1} )</td>
<td>( 2.55 \times 10^{-1} )</td>
</tr>
<tr>
<td>Central retinal artery caliper</td>
<td>Continuous</td>
<td>EAS</td>
<td>6,976</td>
<td>63</td>
<td>-0.320</td>
<td>( 1.39 \times 10^{-4} )</td>
<td>( 9.07 \times 10^{-1} )</td>
<td>( 1.39 \times 10^{-4} )</td>
</tr>
</tbody>
</table>

Ehret, Fereira et al, Nature Genetics 2016

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Enrichment and pathway analyses

1) Using DNase hypersensitivity sites of 123 cell types: enrichment of marks at BP-associated SNPs in microvascular endothelial cells

2) Expression data from GTEx: enrichment in arterial tissues.

Figure 5 Tissue-specific eQTL analysis of 51 tissues. The two outlier tissues, accounting for total eQTL count, are labeled: (a,b) Total eQTL counts versus P-values are shown by tissue when identifying eQTLs by locus (a) and sentinel variant (b).

Ehret et al., Nature Genetics 2016
Hoffmann, Ehret et al., Nature Genetics 2016
Summary epidemiology and genetics of BP

- HTN is quantitatively the most important cardiovascular risk factor and HTN is still poorly controlled.
- The genetic underpinnings of primary hypertension are hundreds to thousands of SNPs with small individual effect sizes.
- The kidney might not be a major causal organ for primary hypertension, is it the vascular endothelium?
- Currently genomics *cannot* guide treatment for primary hypertension.
“direct to consumer” DNA testing

Others: Navigenics, deCODEme, ...
23andMe

- Mission: “23andMe's mission is to be the world's trusted source of personal genetic information”
- About 200’000 SNPs are genotyped
- Cost ~100CHF

Here's what you do:

1. Order a kit from our online store.
2. Register your kit, spit into the tube, and send it to the lab.
3. Our CLIA-certified lab analyzes your DNA in 6-8 weeks.
4. Log in and start exploring your genome.
23andMe: Company destiny

The FDA has now severely reduced the amount of diagnostic information that 23andMe can provide.
Overall summary

• Complex genetic traits are generally not ready for diagnostic laboratory testing.
• There are appealing and interesting other avenues how to use genetic information on complex traits for our patients.
Acknowledgments

**Consortia and studies**
International Consortium for Blood Pressure Genome-Wide Association Studies (ICBP)

CardioMetabochip – ICBP consortium

Cohorts for Heart and Aging Research in Genome Epidemiology (CHARGE) – BP consortium

Atherosclerosis Risk in Communities Study (ARIC)  
(PI for BP genomics: Aravinda Chakravarti)

SKIPOGH study (PI: Prof. Murielle Bochud)

Prof. Aravinda Chakravarti and Prof. François Mach

**Personal grant support**
Swiss National Foundation  
Geneva University Hospitals  
Fondation pour Recherches Médicales  
NHLBI