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Swiss Tropical and Public Health Institute
Schweizerisches Tropen- und Public Health-Institut
Institut Tropical et de Santé Publique Suisse

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Interaction of oxidative stress pathway genes with particulate matter and tobacco smoke on the course of airflow obstruction during 11 years

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Background

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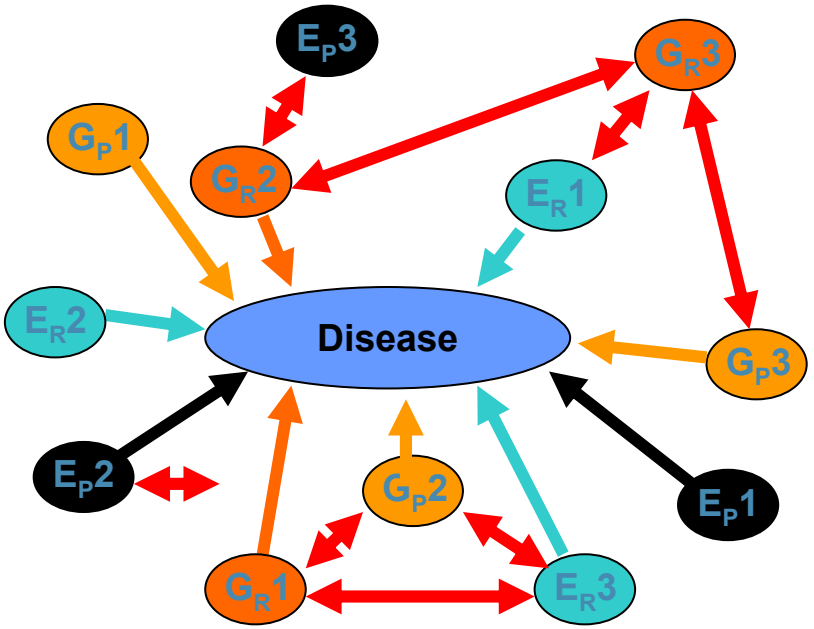
-Chronic obstructive lung disease (COPD) and airway obstruction have high public health impact

-Main risk factor smoking, but other exposures like ambient air pollution contribute substantially

-Oxidative stress & inflammation:
> induced by air pollution and tobacco smoke
> hypothesized etiological pathway for COPD

-Individual risk:
> determined by genetic susceptibility in interaction with environm. factors

Polygenic model of Complex disease:



G: gene, E: envirnoment, P: protective, R: risk factor

->In contrast, genome-wide studies focused on main effects of single nucleotide polymorphisms/mutations (SNPs) so far

Goal

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-To assess how ambient air pollution contributes to the development of airway obstruction and

-To contrast its gene-environment interactions to those of tobacco smoke

By

-assessing gene-environment interactions between oxidative stress-related genes/pathways and PM10/tobacco smoke exposure in the population based SAPALDIA cohort study

thereby

-using pathway-based analysis techniques to assess the involvement of whole genes and pathways

Methods

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The SAPALDIA Study

SAPALDIA 1 **1991**

- Health interview

Measurements:

- Lung function
- Methacholine Test
- Skin prick test
- Height, Weight
- Endexspiratory CO

Blood sampling:

- Phadiatop
- Total IgE

n=9651

Follow-up residential history

SAPALDIA 2 **2002**

- Health interview

Measurements:

- Lung function
- Methacholine Test
- Holter (age 50-73)
- Blood pressure
- Height, Weight
- Endexspiratory CO

BIOBANK:

- Phadiatop
- Specific IgE
- Genetic markers
- CV risk-factors

n=8047

Air pollution monitoring

1991 2001-03

Methods

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Analysis

Study sample: **650 non-asthmatic SAPALDIA adults** with gen.-wide data and complete covariate information

Outcome: **FEV1/FVC-decline** in 11yrs (pre-bronchodilation spirometry)

Genes: **152 oxidative stress genes** (12679 SNPs), **14 pathways**

Exposure: individual **interval PM10- or packyears** exposure in 11yrs

Analysis: 1. SNP-level interaction analysis:

$$FEV1/FVC\text{-}decline = \alpha + \beta_1 * packyears_{interval} + \beta_2 * PM10_{interval} + \beta_3 * SNP_{additive} + \beta_4 * SNP_{additive} * exposure +$$

controlling for $packyears_{baseline}$, sex, age, height, study area and principal components of population ancestry

where *exposure* = $PM10_{interval}$ or $packyears_{interval}$

2. derive gene- and pathway level interaction p-values from SNP-p-values using ARTP method [1)]
3. Comparison of emerging genes for PM10 and packyears exposure (and interaction effect sizes on SNP-level)

1) Yu K et al. *Genet Epidemiol* 2009 ; 33(8): 700–709.

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Genes:

AATF	ABCC1	AGT	AGTR1	AKR1A1	AKR7A2	AKR7A3	ALOX12
AOX1	ARNT	ATOX1	BCL2	BCL2L1	CASP6	CAT	CCL5
CDK1	CDKN1A	CHUK	COL1A1	CP	CRISP2	CYBA	CYGB
CYP1A1	CYP1A2	DHCR24	DHRS2	DUSP1	EGFR	EP300	EPHX1
EPHX2	EPX	ERCC1	FMO2	FOS	FOSL1	GCLC	GCLM
GLRX	GLRX2	GLRX3	GLRX5	GPX1	GPX2	GPX3	GPX4
GPX5	GPX6	GPX7	GPX8	GRB2	GSR	GSS	GSTCD
GSTK1	GSTM1	GSTM2	GSTM3	GSTM4	GSTM5	GSTO1	GSTO2
GSTP1	GSTT1	GSTT2	GSTZ1	HMOX1	HMOX2	HP	IDH1
INSR	JAK2	JUN	KEAP1	LPO	MAP2K1	MAPK14	MGST1
MGST2	MGST3	MPO	MSRA	MT2A	NAPRT1	NCF2	NDUFA12
NDUFA13	NDUFA6	NDUFS1	NDUFS2	NDUFS3	NDUFS4	NDUFS8	NFE2L2
NFKB1	NOS1	NOS2	NOS3	NOX3	NOX4	NOX5	NOXO1
NQO1	NQO2	OGG1	OXR1	PARK2	PARK7	PLA2G4A	PLCB1
PLCG1	PNKP	PPP2CB	PRDX1	PRDX2	PRDX3	PRDX5	PRDX6
PRKCA	PSEN1	PSMB5	PTGS1	PTGS2	PTK2B	PXDN	PYCR1
RAC1	RAC2	RELA	RIPK1	SCARA3	SEPP1	SLC23A2	SNCA
SOD1	SOD2	SOD3	SRXN1	STAT1	STK25	TGFBR2	TLR4
TP53	TPO	TXN	TXN2	TXNIP	TXNRD1	TXNRD2	UCP2

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Pathways:

- Apoptosis Signaling
- Arachidonic Acid Metabolism
- Aryl Hydrocarbon Receptor Signaling
- Metabolism of Xenobiotics by Cytochrome P450
- fMLP Signaling in Neutrophils
- Glutathione Metabolism
- IL-6 Signaling
- Methane Metabolism
- Mitochondrial Dysfunction
- NF-κB Signaling
- Production of Nitric Oxide and Reactive Oxygen Species in Macrophages
- NRF2-mediated Oxidative Stress Response
- Oxidative Phosphorylation
- Xenobiotic Metabolism Signaling

Results

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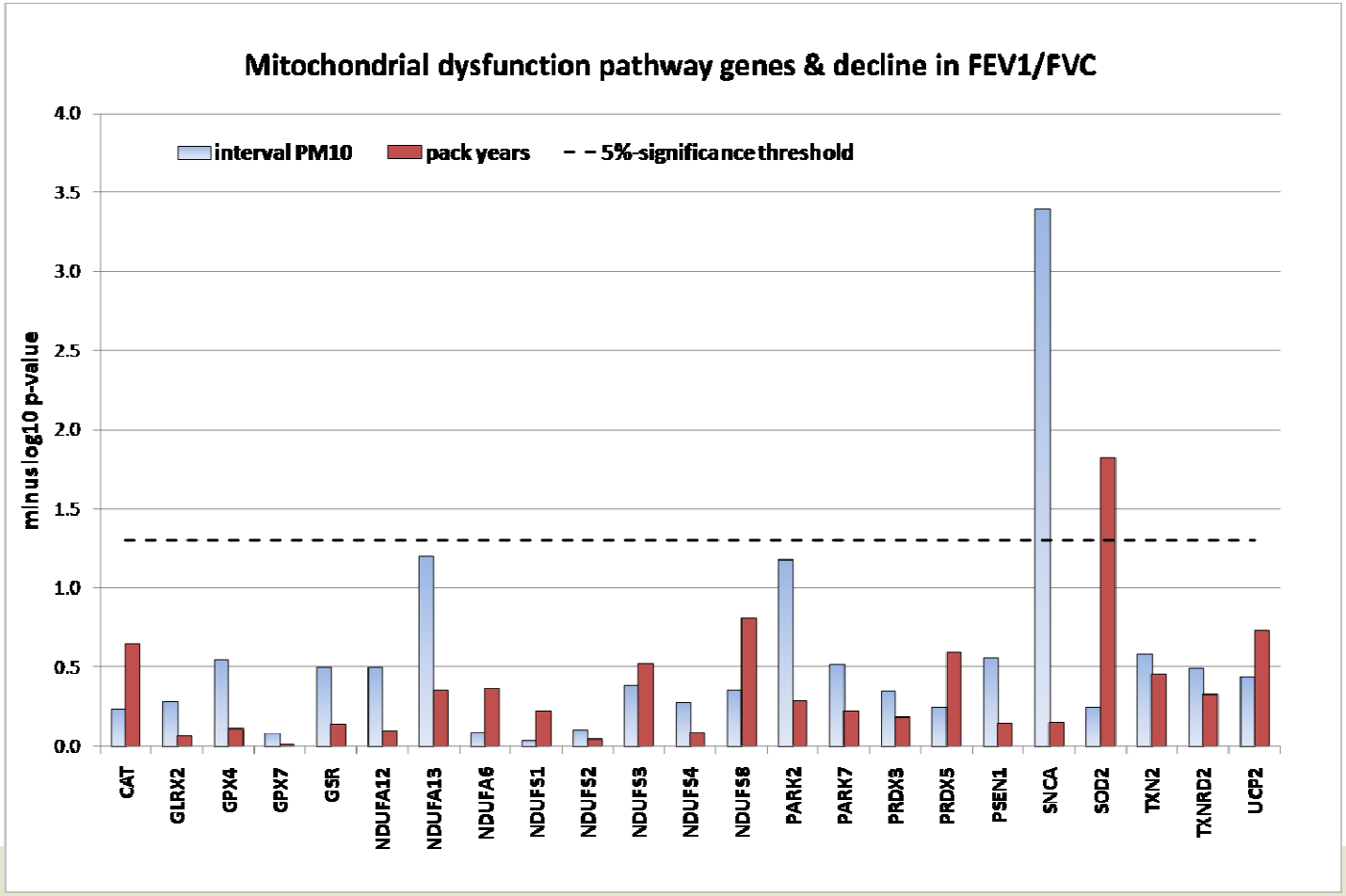
-No pathway interaction with packyears exposure
-Interaction signal for pathway „mitochondrial dysfunction“ with PM10 exposure ($P_{interaction}$=0.017)



Results

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Gene-level interaction signals

Genes with nominally significant interaction signals (α=0.05)

interval PM10 exposure [gene(p-value)]		Packyears exposure [gene(p-value)]	
ALOX12	(p=0.012)	HMOX2	(0.048)
CHUK	(0.035)	MAP2K1	(0.019)
CRISP2* (p=0.0003)		NFKB1	(0.022)
EPX	(0.040)	**PSMB5**	**(p=0.003)**
ERCC1	(0.007)	SOD2	(0.015)
GPX5	(0.039)		
LPO	(0.018)		
MPO	(0.039)		
SNCA* (p=0.0004)			

*CRISP2 significant after Bonferroni correction for 152 genes (α=3.3E-04), SNCA marginally

Results

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SNP-level estimates

Exposure	Gene	SNP	All1	Freq All1	$Beta_{int}$ (SE), P [1)]	$Beta_{SNP}$ (SE), P [1,2)]	$Beta_{exp}$ (SE), P [1)]
PM10	***SNCA***	Rs2035268	G	0.05	**-3.8** (0.8), *2.54E-06* [3)]	-0.7 (0.6), *0.254*	-0.2 (0.7), *0.786*
(IQR 83.4 ug/m3 * y)	***CRISP2***	**rs360563**	C	0.50	**-1.1** (0.3), *3.78E-05*	0.0 (0.3), *0.975*	0.6 (0.7), *0.375*
packyrs	***PSMB5***	rs12590429	A	0.09	**-3.8** (0.9), *1.06E-05*	0.3 (0.5), *0.540*	-0.5 (0.5), *0.265*
(IQR 9.8 PY)							

[1)] Beta-estimates: % FEV1/FVC-decline over 11yrs per allele and/or exposure contrast of 1 IQR.
[2)] Additive SNP model
[3)] **Significant in SNP-level analysis** correcting for 12679 tests (α=3.9E-06)

-similar effect sizes for PM10 and packyears exposure, but **unsuccessful replication** attempt for **rs360563** in CRISP2 in the remainder of SAPALDIA population (n=3320, p=0.63)

-(percent explained variability up to 19% in models including all top-SNPs of nominally significant genes)

Conclusion

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-first pathway analysis of gene-environment interaction on lung function decline employing a very broad set of oxidative-stress related candidate genes

-suggestive evidence that different genes could be involved in mediating effects of PM10 and tobacco smoke exposure

-Gene & pathway based analysis technically feasible, potentially yields additional hits beyond pure SNP-level analysis

-Limitations:

- -limited sample size (genome-wide genotyping resource intense)
- -limited pathway coverage (pathway signals gene-driven)
- -ARTP pathway method accumulates SNP-level interaction signals along gene (gene along pathway), complexity of biological signals likely much higher
- -likewise, studied exposure estimates only proxies for underlying biologically active substances

->Need for large scale studies with detailed genetic, environmental and phenotypic characterization

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SAPALDIA

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Scientific team: *JC Barthélémy, W Berger, R Bettschart, A Bircher, G Bolognini, O Brändli, C Brombach, M Brutsche, L Burdet, M Frey, U Frey, MW Gerbase, D Gold, E de Groot, W Karrer, R Keller, B Knöpfli, B Martin, D Miedinger, U Neu, L Nicod, M Pons, F Roche, T Rothe, E Russi, P Schmid-Grendelmeyer, A Schmidt-Trucksäss, A Turk, J Schwartz, D. Stolz, P Straehl, JM Tschopp, A von Eckardstein, E Zemp Stutz.*

Scientific team at coordinating centers: *M Adam, E Boes, PO Bridevaux, D Carballo, E Corradi, I Curjuric, J Dratva, A Di Pasquale, L Grize, D Keidel, S Kriemler, A Kumar, M Imboden, N Maire, A Mehta, F Meier, H Phuleria, E Schaffner, GA Thun, A Ineichen, M Ragettli, M Ritter, T Schikowski, G Stern, M Tarantino, M Tsai, M Wanner.*

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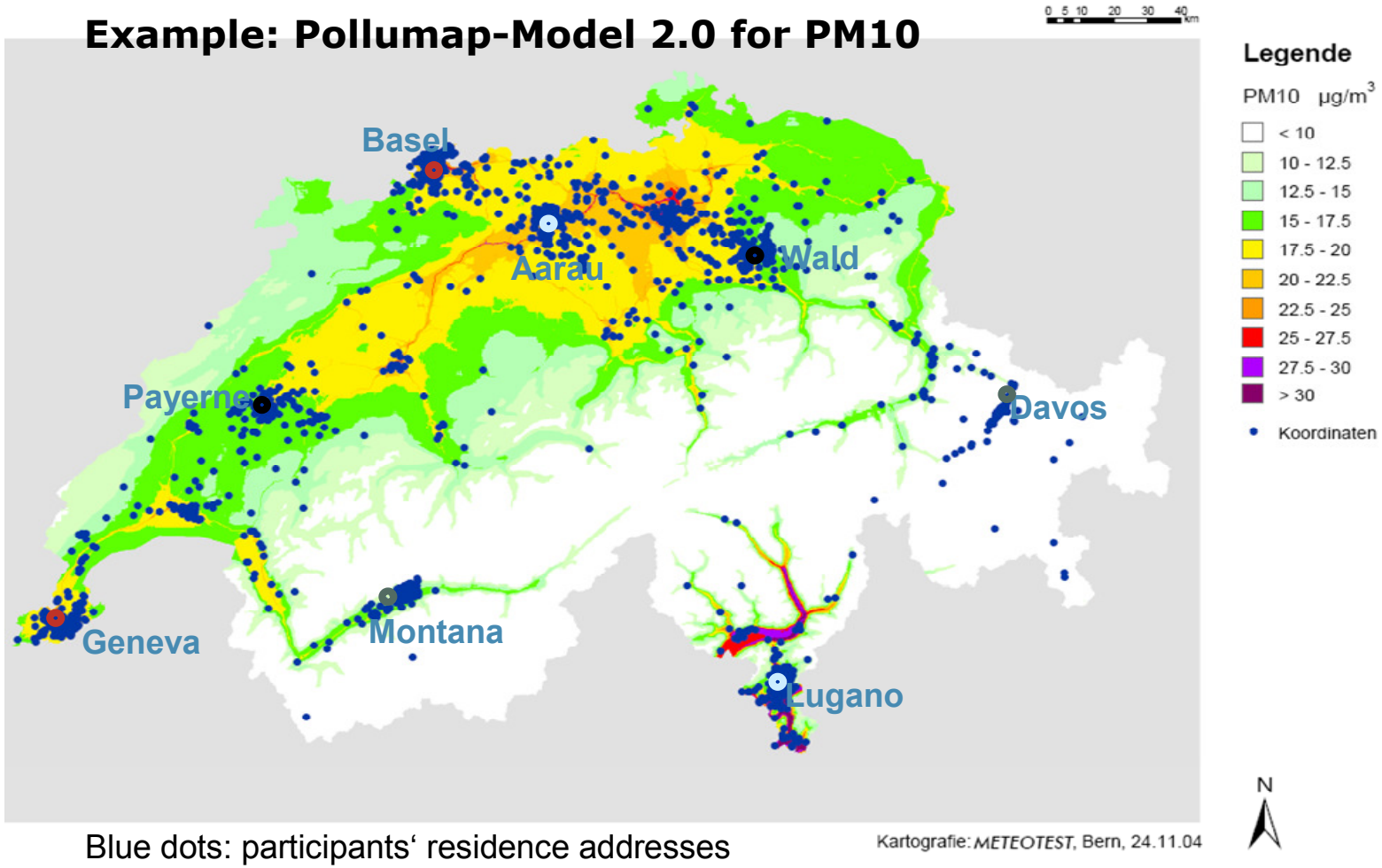
Methods

AIR POLLUTION MODELLING

Example: Pollumap-Model 2.0 for PM10



Blue dots: participants' residence addresses

Methods

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GENETIC DATA

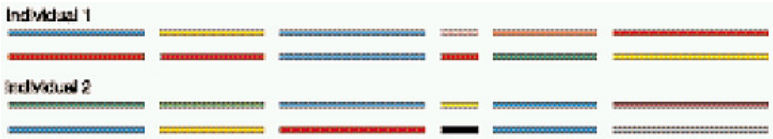
DNA

-high quality DNA samples for over 6000 participants

Genome-wide genotyping & imputation

-GABRIEL project: genotyping of ~550‘000 single nucleotide polymorphisms (SNPs) per person

-imputation to ~2.5 Mio SNPs standard reference population of European origin (HapMap CEU)



1)

-Genome wide data for 878 non-asthmatic participants

Candidate genes & pathways related to oxidative stress

-selection of **152 genes** (**12679 SNPs**) from public databases
-genes mapping to **14 pathways** (37 genes not mapped)

1) Adapted from *The mosaic that is our genome. Pääbo S. Nature 2003;421:409*

Methods

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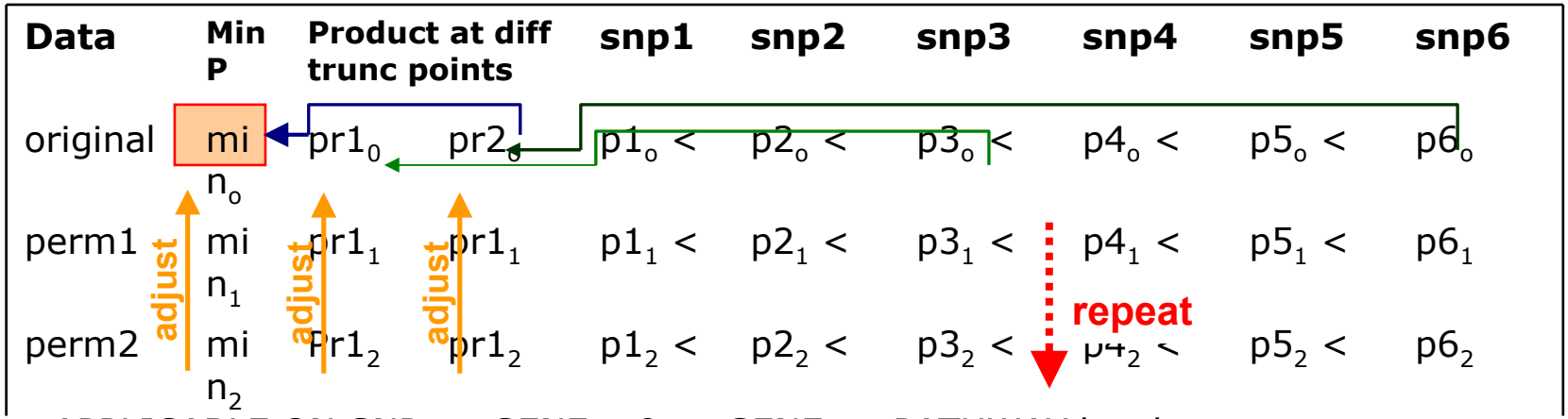
Adaptive Rank Truncation Product method [1)]

TECHNIQUE

1. Odering of p-values from single SNP analysis in ascending order
2. Calculate **products** of ranked p-values at different truncation points (gene length)
3. **Adjust** product p-values using permutation distribution
4. **Pick minimum** of adjusted products and readjust -> **PGene**



APPLICABLE ON SNP -> GENE & GENE -> PATHWAY level

Use of PERMUTATION allows application in a wide range of analysis settings

RESOURCE requirements (time, IT) ~modest, as only 1 level of permutations done

1) Yu K et al. *Genet Epidemiol* 2009 ; 33(8): 700–709.