Genetic Variation at the N-acetyltransferase (*NAT*) Genes in Global Populations

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IGERT Integrative Graduate Education and Research Traineeship
NATIONAL RECRUITMENT PROGRAM

Project Aims

•To identify and characterize nucleotide diversity at the **N-acetyltransferase (*NAT*) genes** located on chromosome 8 in a panel of globally diverse human populations, with specific focus on Africa.

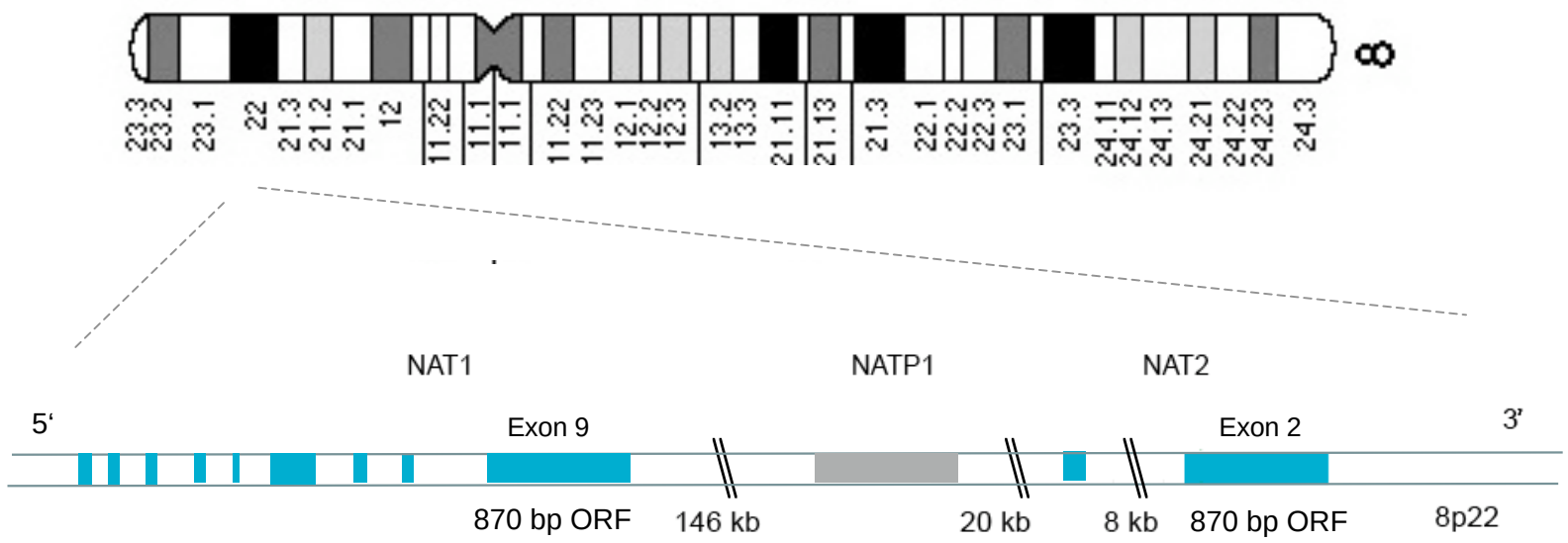
--*NAT* genes are classified as **Drug Metabolizing Enzyme (DME)** loci because of their role in the metabolism of pharmaceuticals

--**DMEs** likely played an important role in detoxification of plant metabolites and other components of diet from which drugs ultimately derive

•To characterize the evolutionary forces (*e.g.* mutation, genetic drift, and natural selection) that have influenced patterns of variation at these loci.

•To identify variants that appear to be targets of natural selection and may be of functional significance

N-Acetyltransferase (*NAT*) Chromosomal Region- 8p22



NAT1 and *NAT2* derive from ancient duplication events

- ~87% sequence homology

NAT1 and NAT2 enzymes-*biotransformation of xenobiotics*

- N-acetylation is a *DETOX* step for aromatic amines

NAT2 metabolizes ISONIZID (INH)

NAT1

•NAT1 is expressed **UBIQUITOUSLY** and at different stages of development

•*NAT1* coding region polymorphisms that **REDUCE** enzyme activity are **RARE** on a population basis

NAT2

•NAT2 expression is confined to the **LIVER**, making it more the “classic” DME

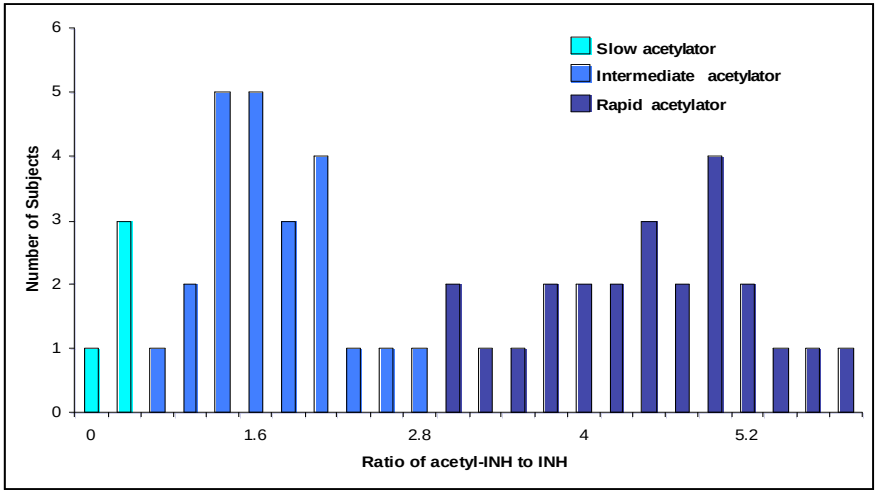
•*NAT2* coding region SNPs that **REDUCE** enzyme activity are **COMMON** across human populations

Barker *et al*. (2006) Pharmacogenetics and Genomics

What is the advantage of having altered acetylation?

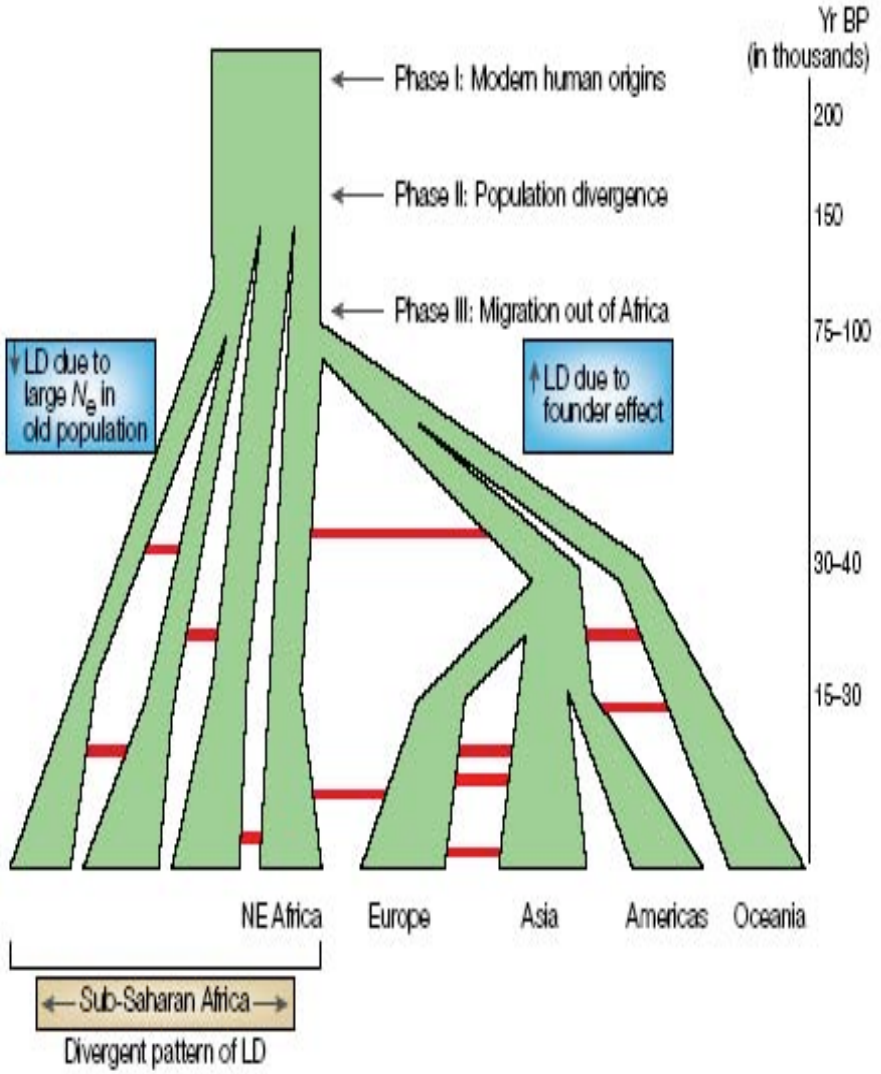
- **NAT2 *SLOW*** acetylators are at risk (**drug induced toxicity, bladder cancer**)

- **NAT2 *RAPID*** acetylators are at risk (**metabolic cancers: colon, stomach**)

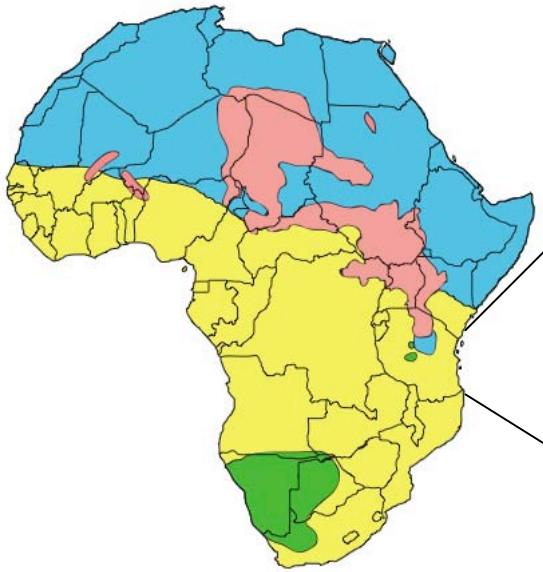


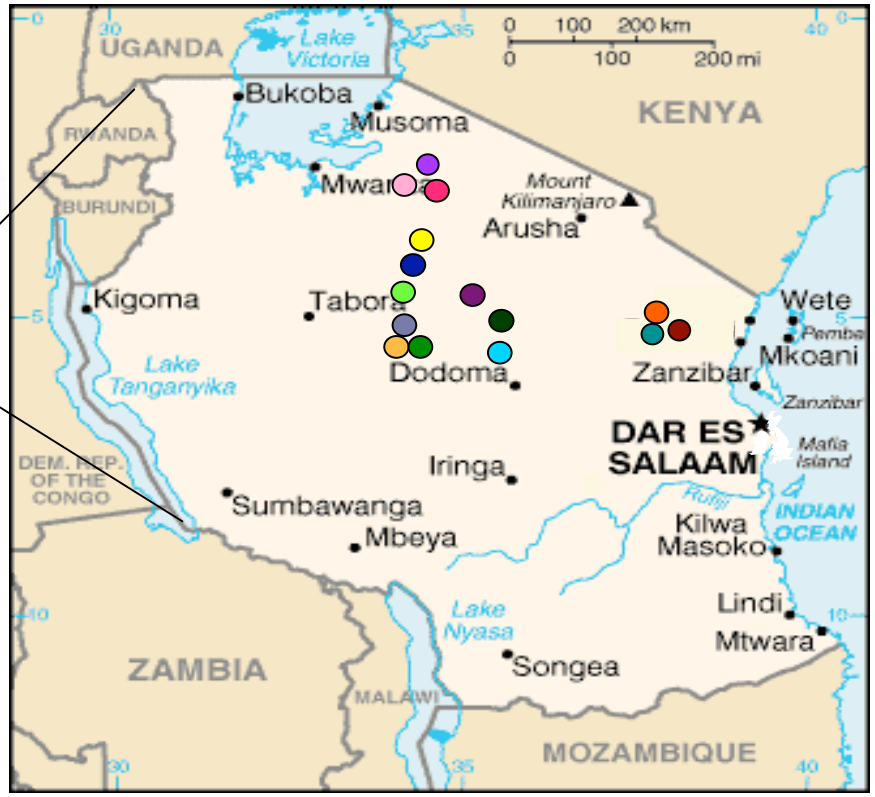
Why *NAT* and Why Africa?

- African Populations-Long Evolutionary History
- Africa- typically *UNDER*-represented
- High incidence of TB in Africa
- Implication to efficacy in drug development



Cultural, Phenotypic, and Subsistence Diversity in Africa



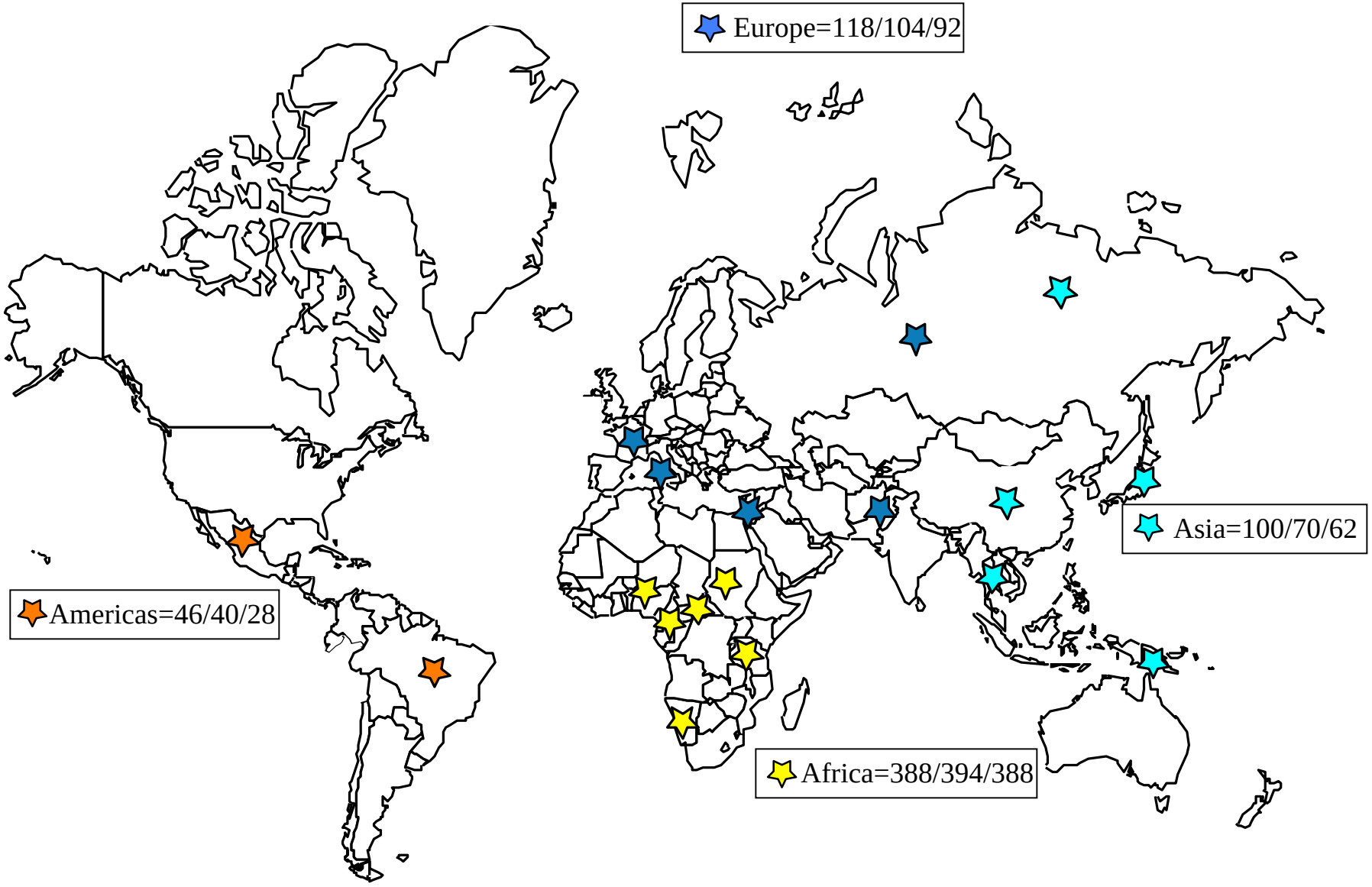


- Hadza
- Sandawe
- Akie
- Dorobo
- Maasai
- Datog
- Burunge
- Iraqw
- WaFiome
- Mbugu
- Gogo
- Turu
- Mbugwe
- Pare
- Sambaa
- Sukuma





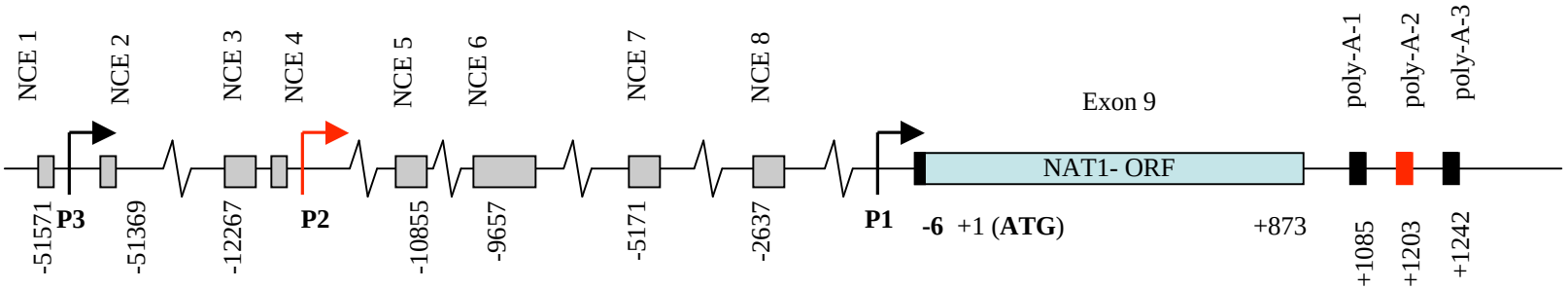

NAT Global Sampling Distribution



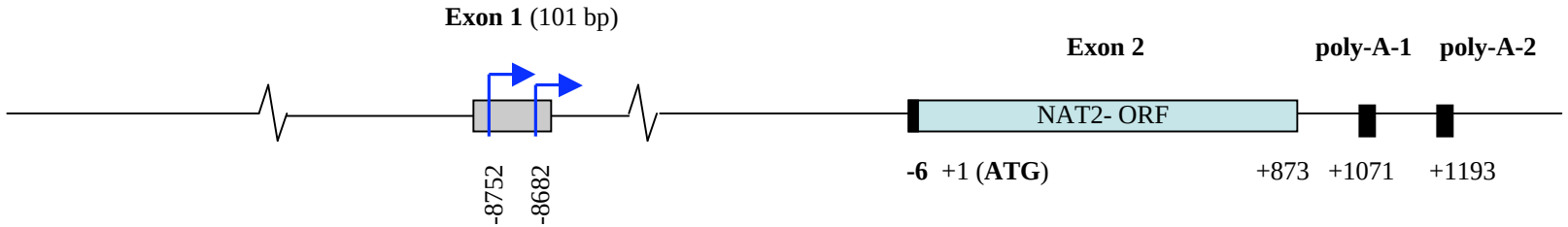
Total (2n): NAT1=652/NATP1=608/NAT2=570

NAT gene structure

NAT1

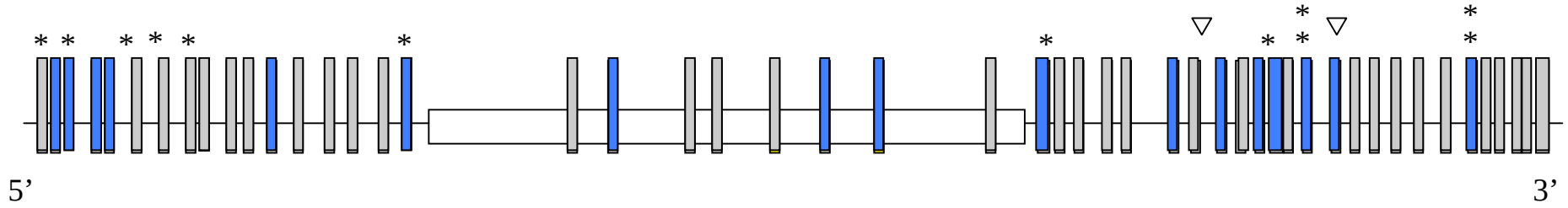


NAT2



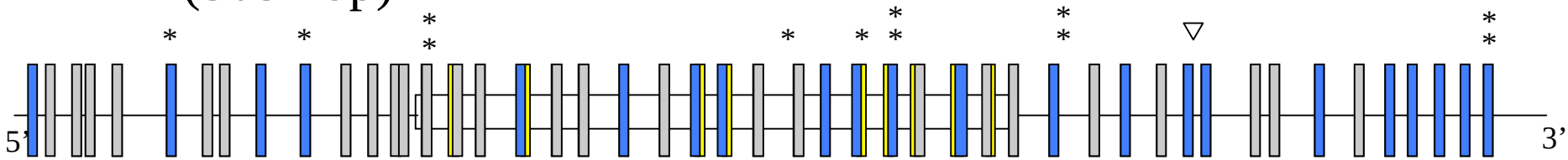
NAT SNPs Identified with Resequencing

*NAT1 (*2856 bp)



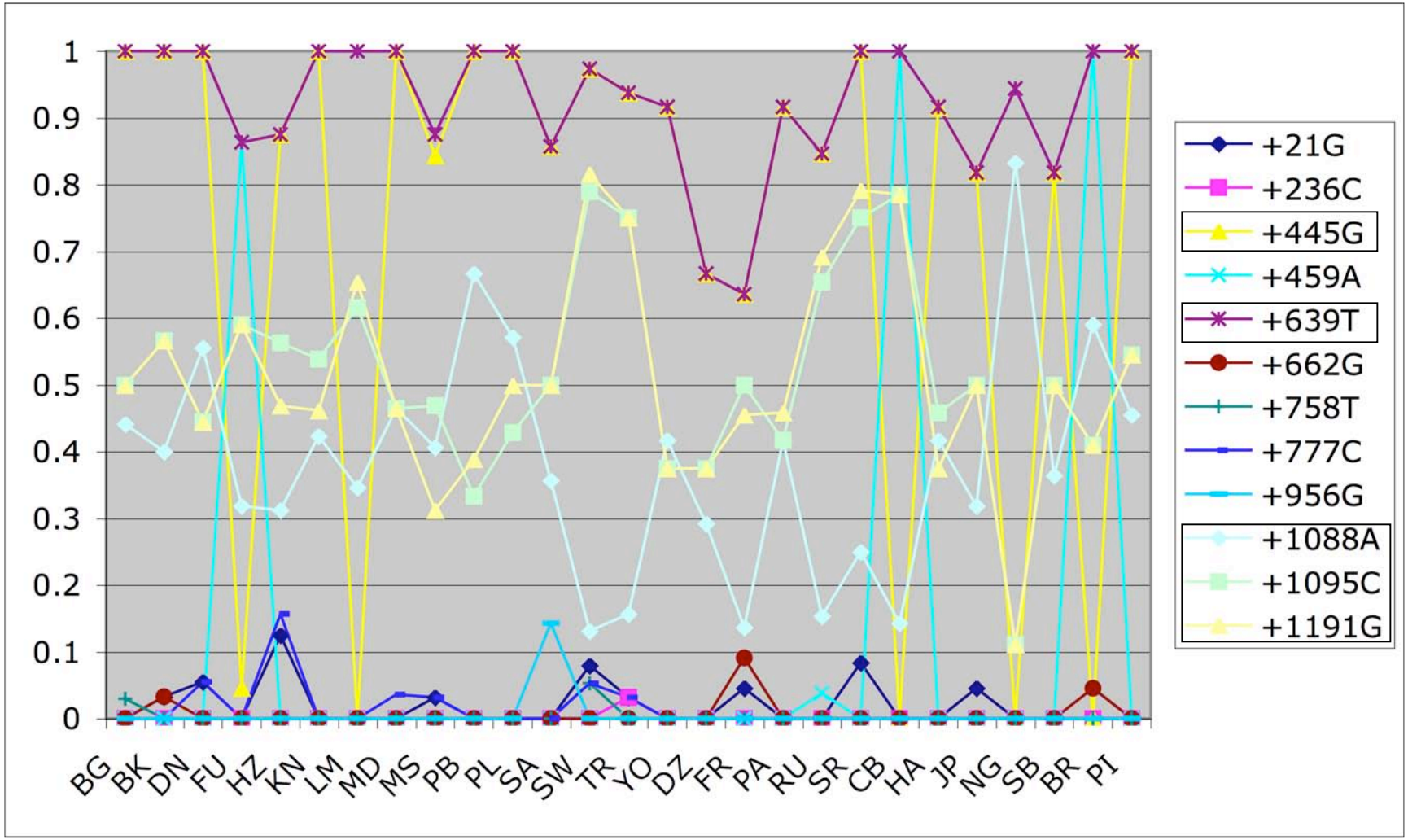
Total SNPs identified=48
Novel SNPs=17
Non-synonymous=2
Silent=46

NAT2 (3031 bp)

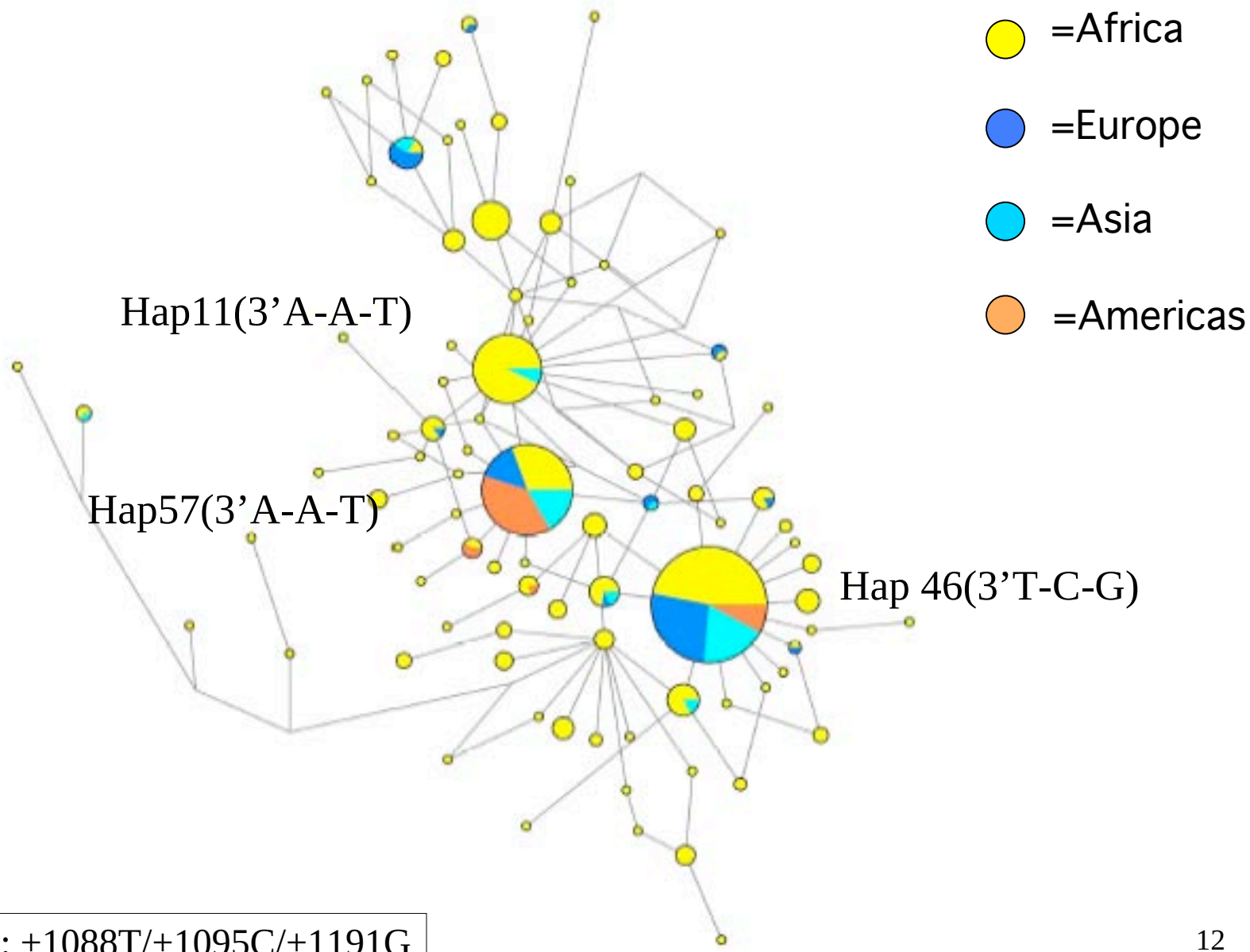


Total SNPs identified=46
Novel SNPs=22
Non-synonymous=15
Silent=31

NAT1 coding and 3’ SNP frequency by population group

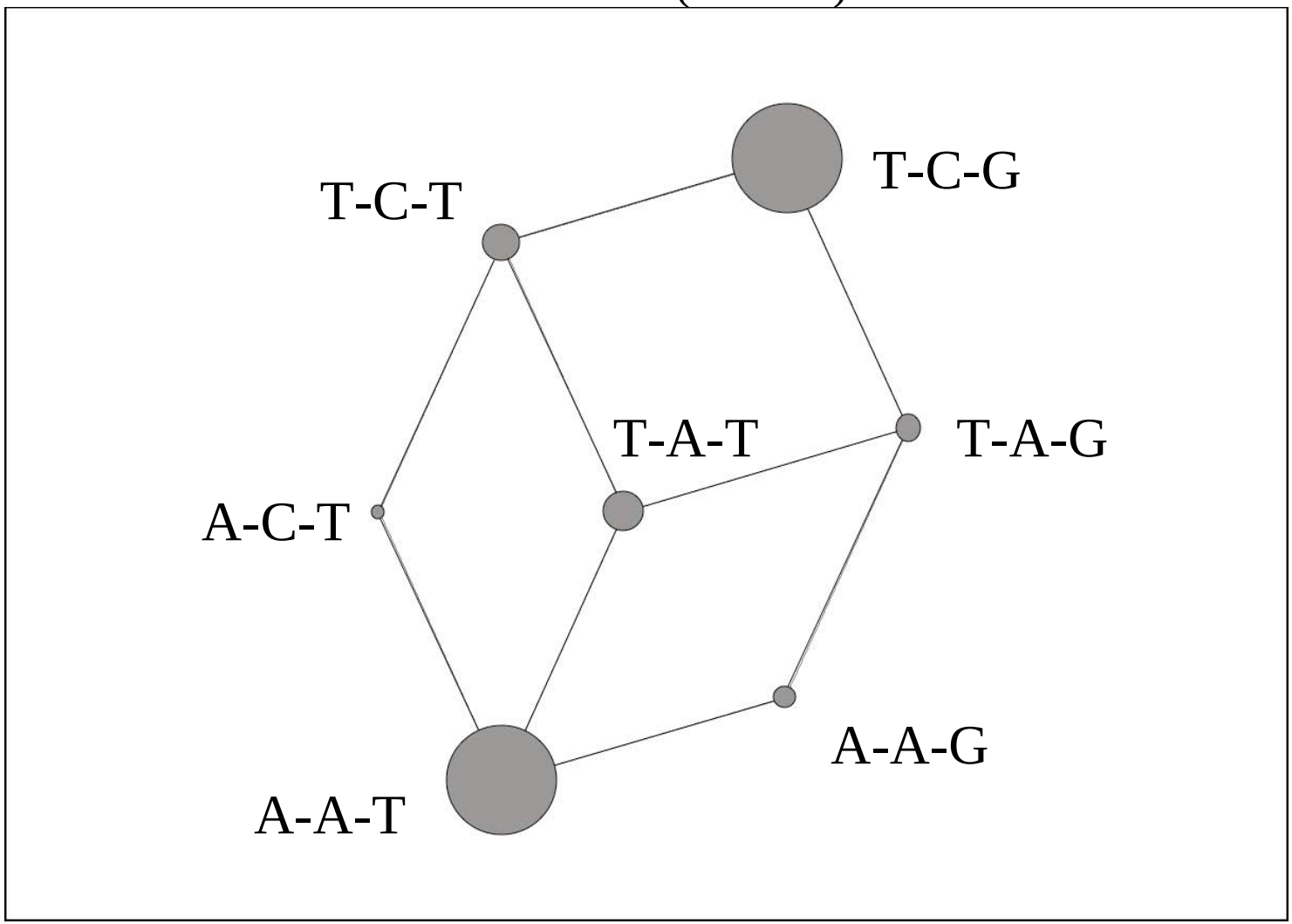


NAT1 Median-Joining Haplotype Network



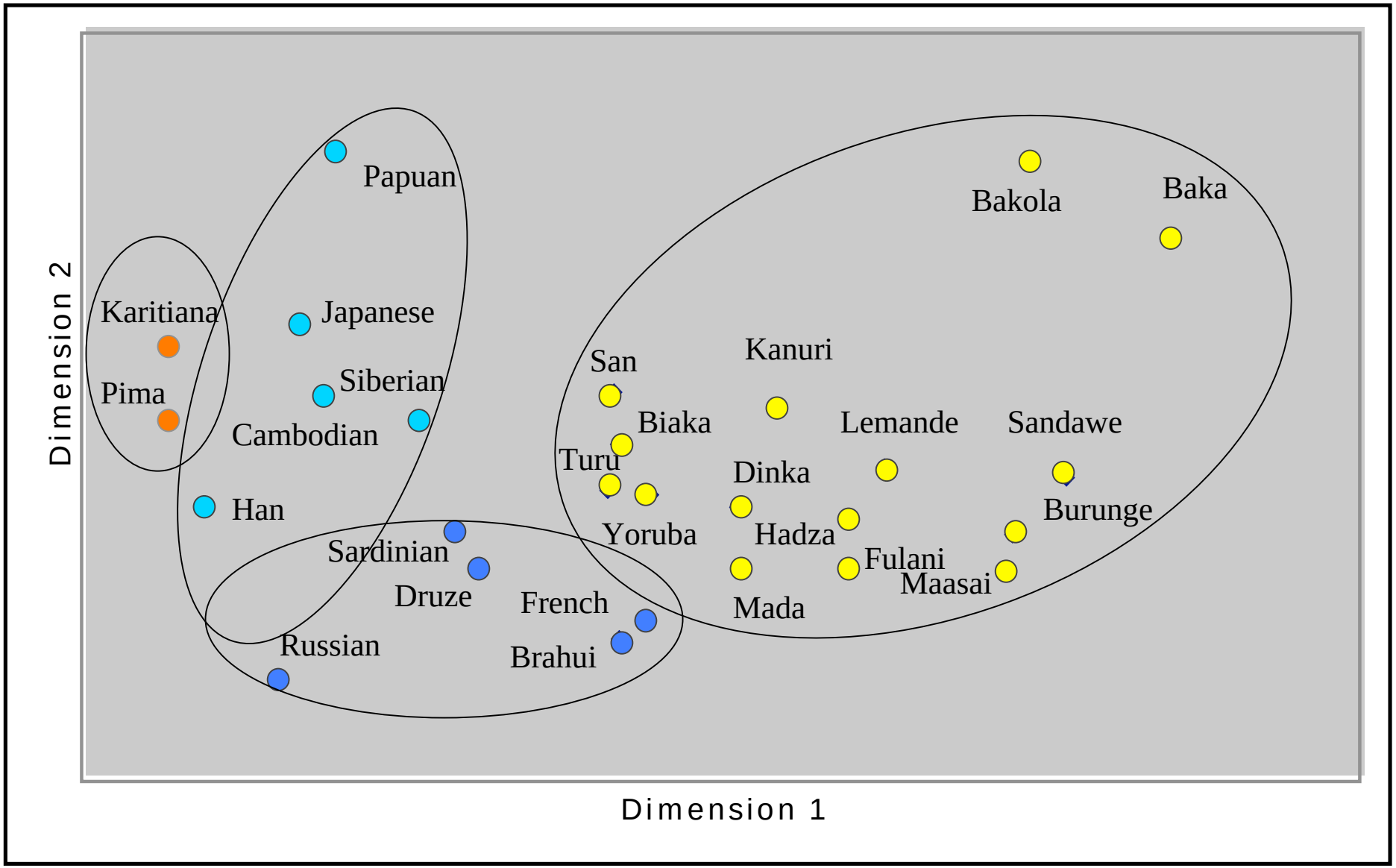
3'SNPs: +1088T/+1095C/+1191G

NAT1 M-J Network (3'SNPs)

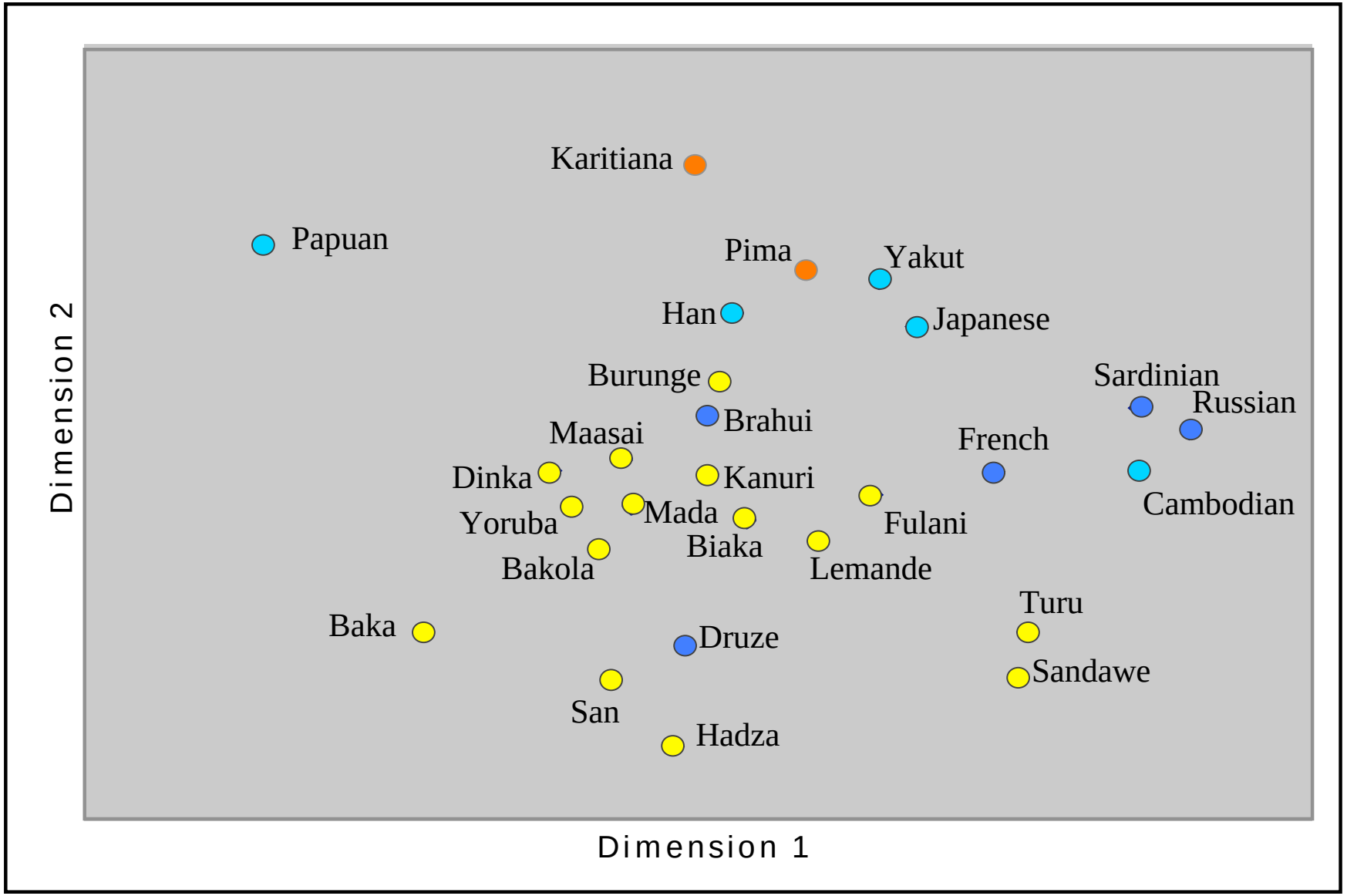


SNP order: 1088-1095-1191

NATP1 Multi-Dimensional Scaling Plot



NAT1 Multi-Dimensional Scaling Plot



Sequence-Based Neutrality Tests of *NAT1*

NAT1 Coding and Flanking Regions (2856 bp)

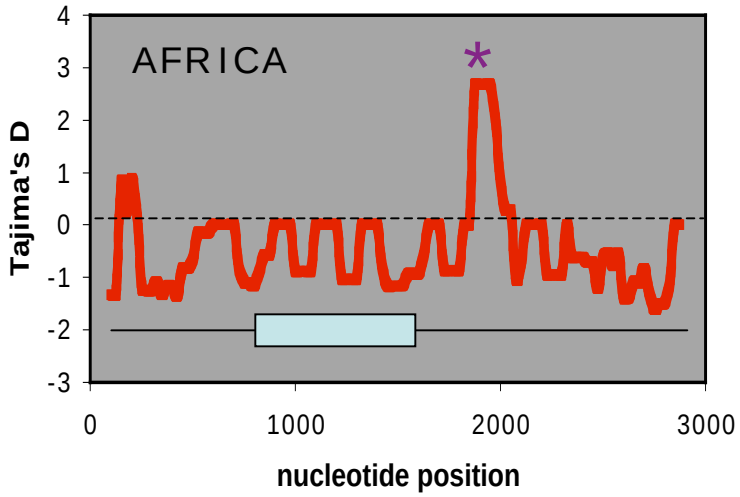
POPULATION	TD	D*	F*	H
Africa	-1.417	0.350	-0.549	-15.340
E Africa	-1.442	-1.357	-1.676	-13.765
W Africa	-1.079	0.921	0.124	-15.072
Hadza	0.246	0.421	0.429	-0.520
Pygmy groups	-0.562	-0.933	-0.952	-3.101
S. African San	-0.249	-0.712	-0.672	-2.637
Europe	-1.300	-1.574	-1.758	-11.113
Asia	-1.374	-4.985	-4.293	-15.057
Americas	0.968	0.481	0.746	-0.955

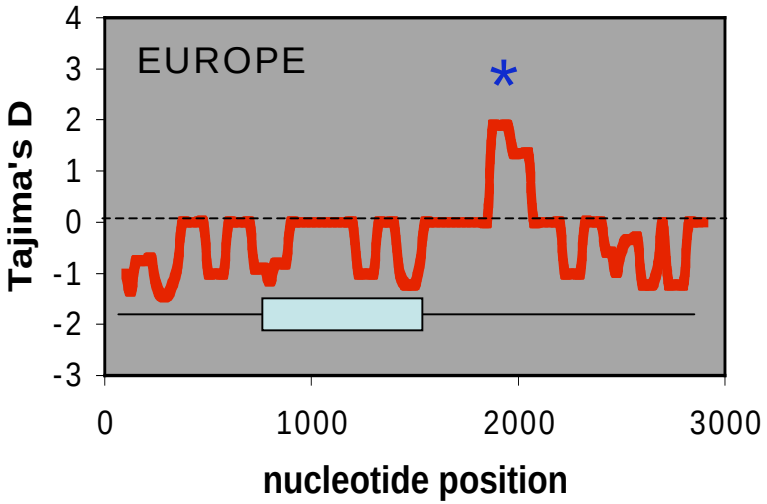
$p<0.05$ $p<0.01$ $p<0.001$

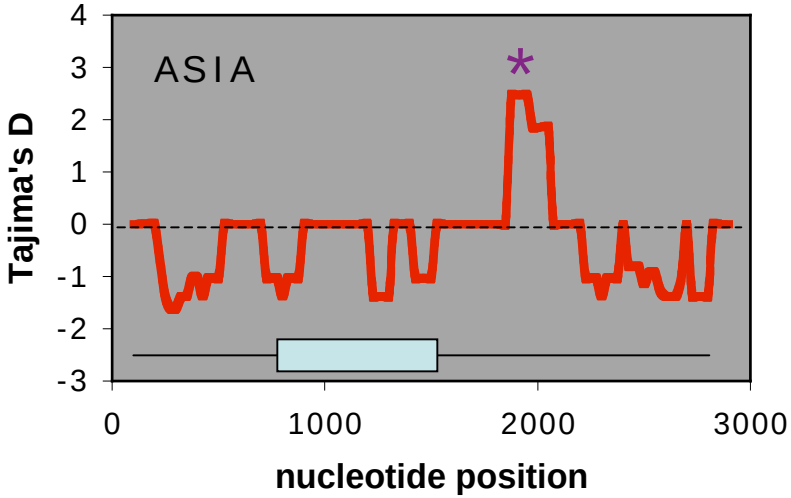
TD (Tajima,1989)
F*/D* (Fu and Li, 1993)
H (Fay and Wu, 2000)

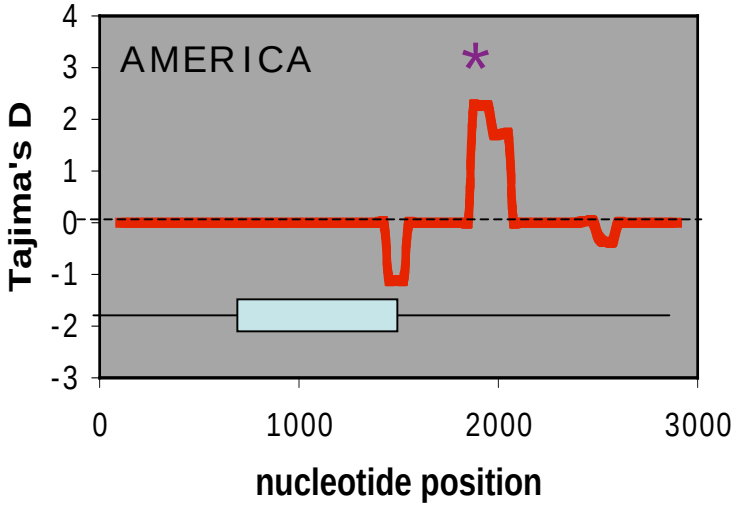
DNAsp 4.20.2 (Rozas and Rozas, 1995)

NAT1 Sliding Window Tajima's D



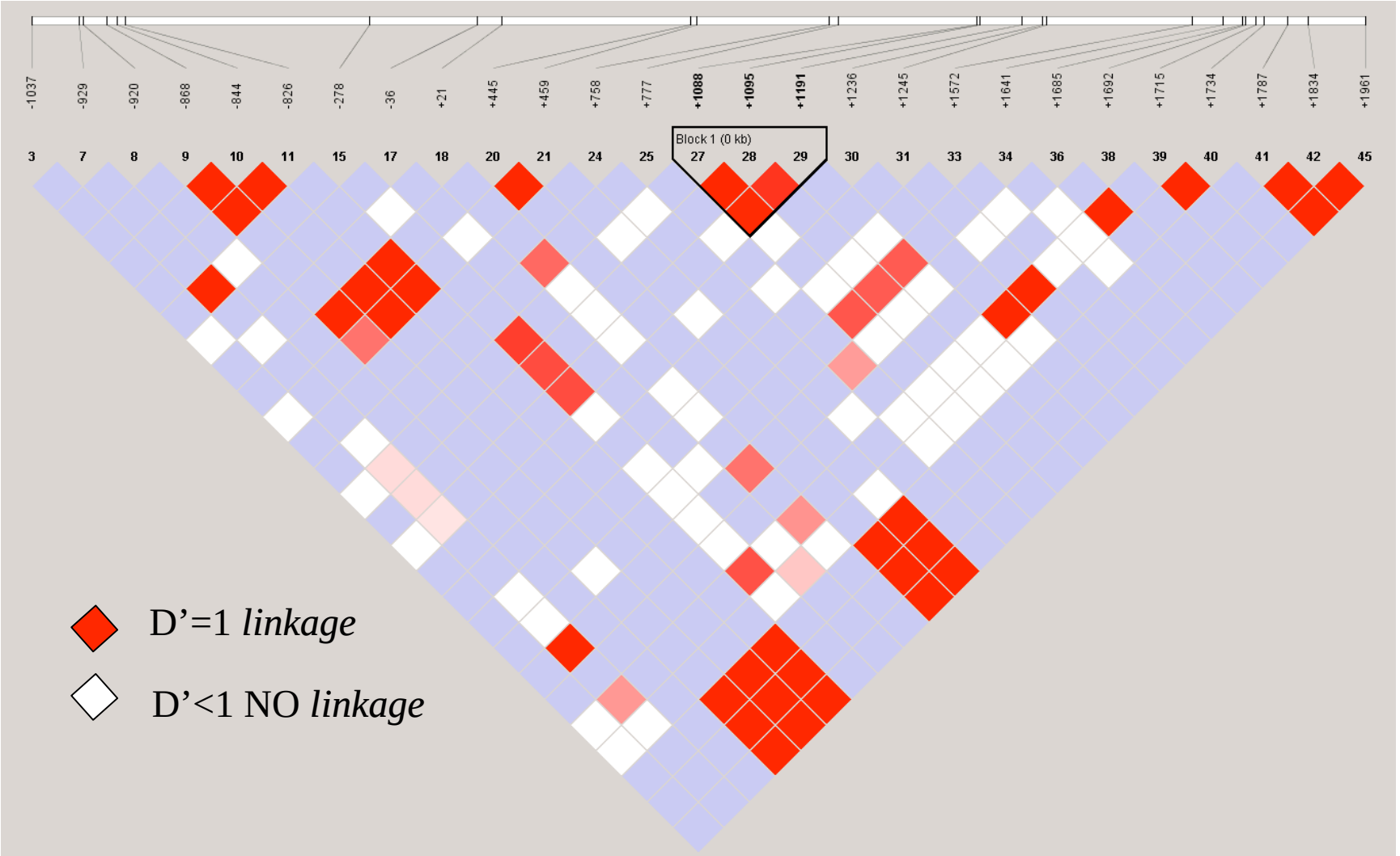






* P<0.10 * P<0.05

NAT1 Africa: Linkage Disequilibrium (LD)/Recombination Plot

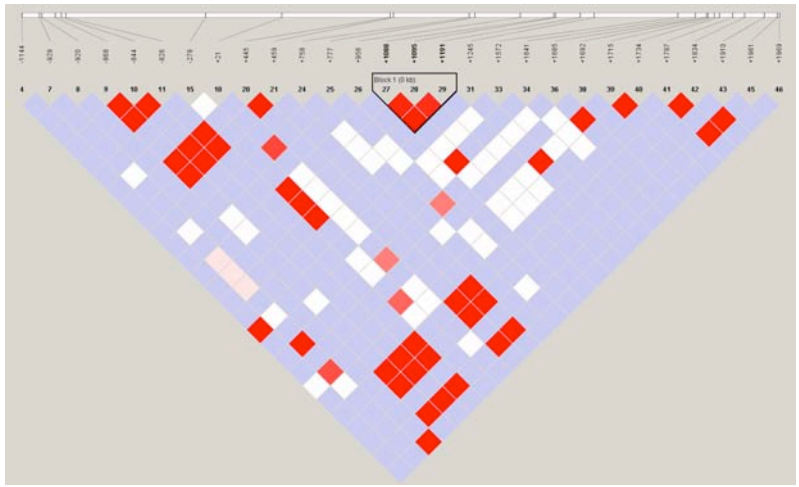


Haploview Barrett, JC *et al*. (2005) Bioinformatics 21(2):263-265**.**

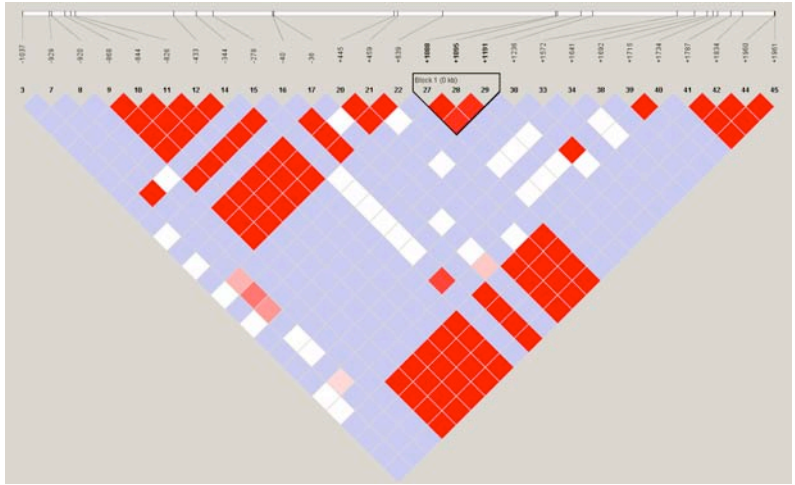
*MAF <0.01 excluded

NAT1

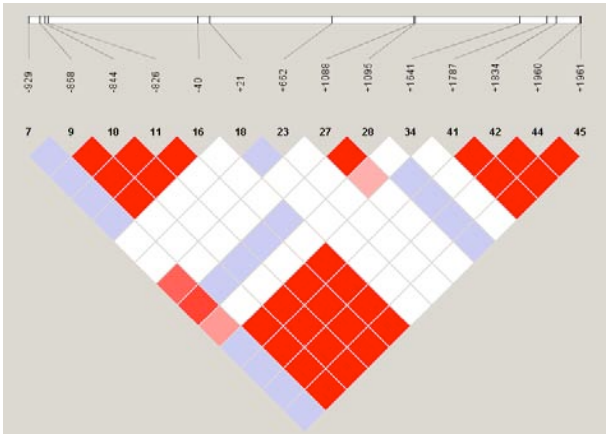
East Africa



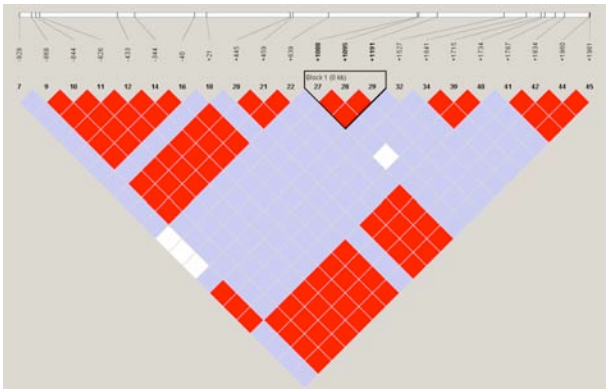
West Africa



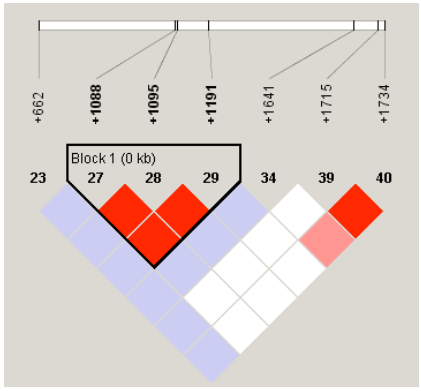
Europe



Asia

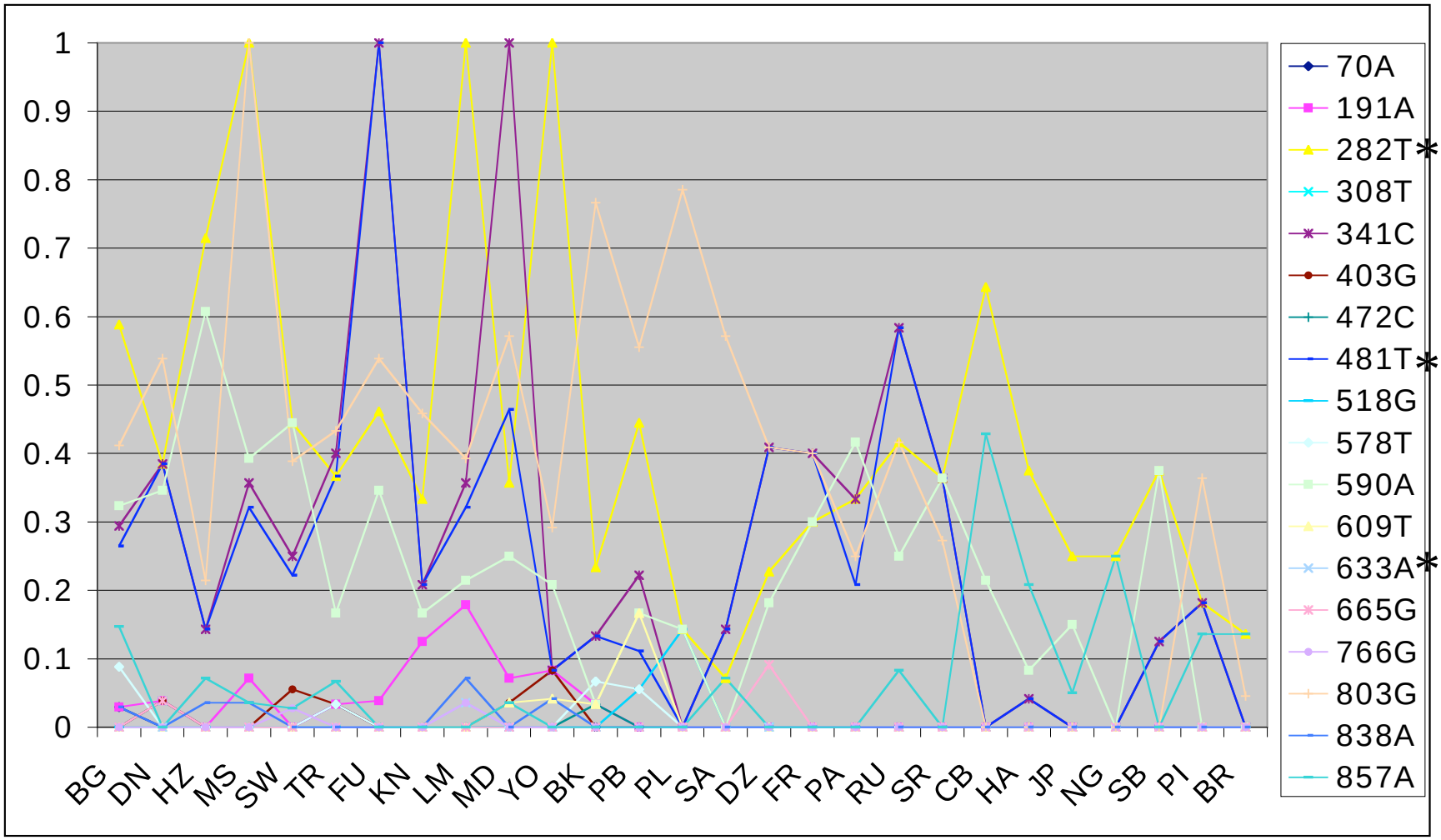


Americas



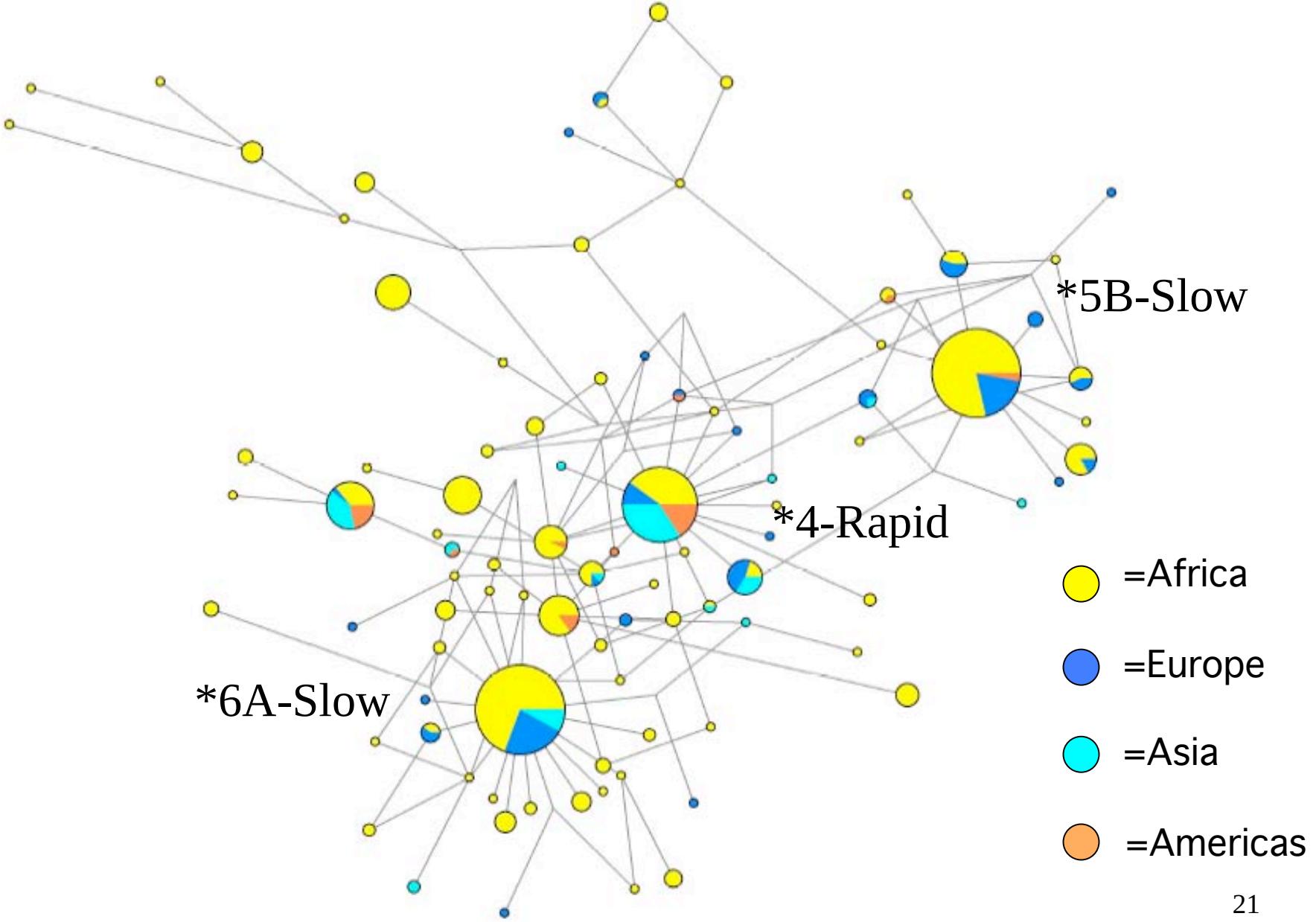
D'=1 *linkage* D'<1 NO *linkage*

NAT2 coding SNP frequency by population group

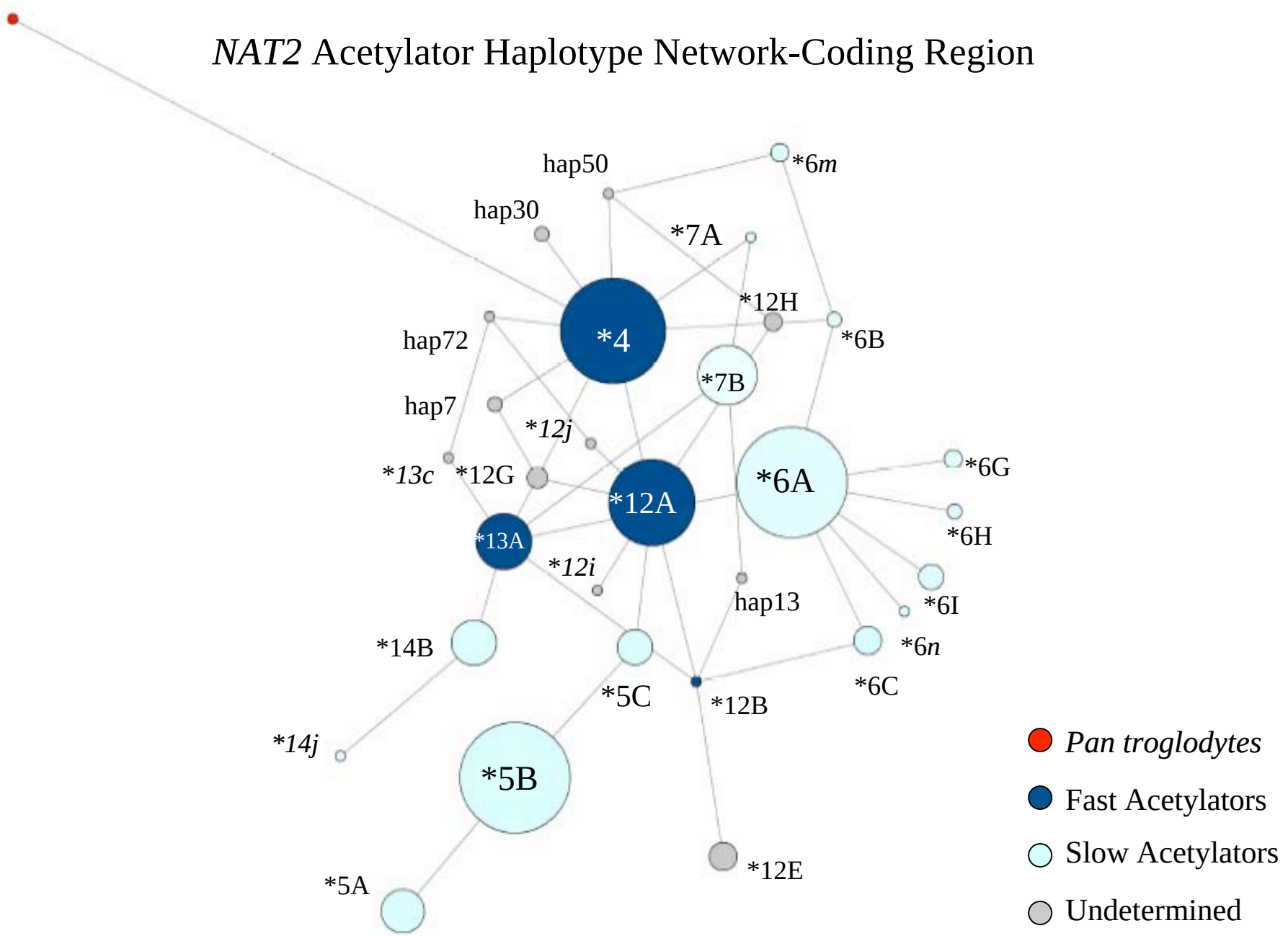


*synonymous

NAT2 Median-Joining Haplotype Network

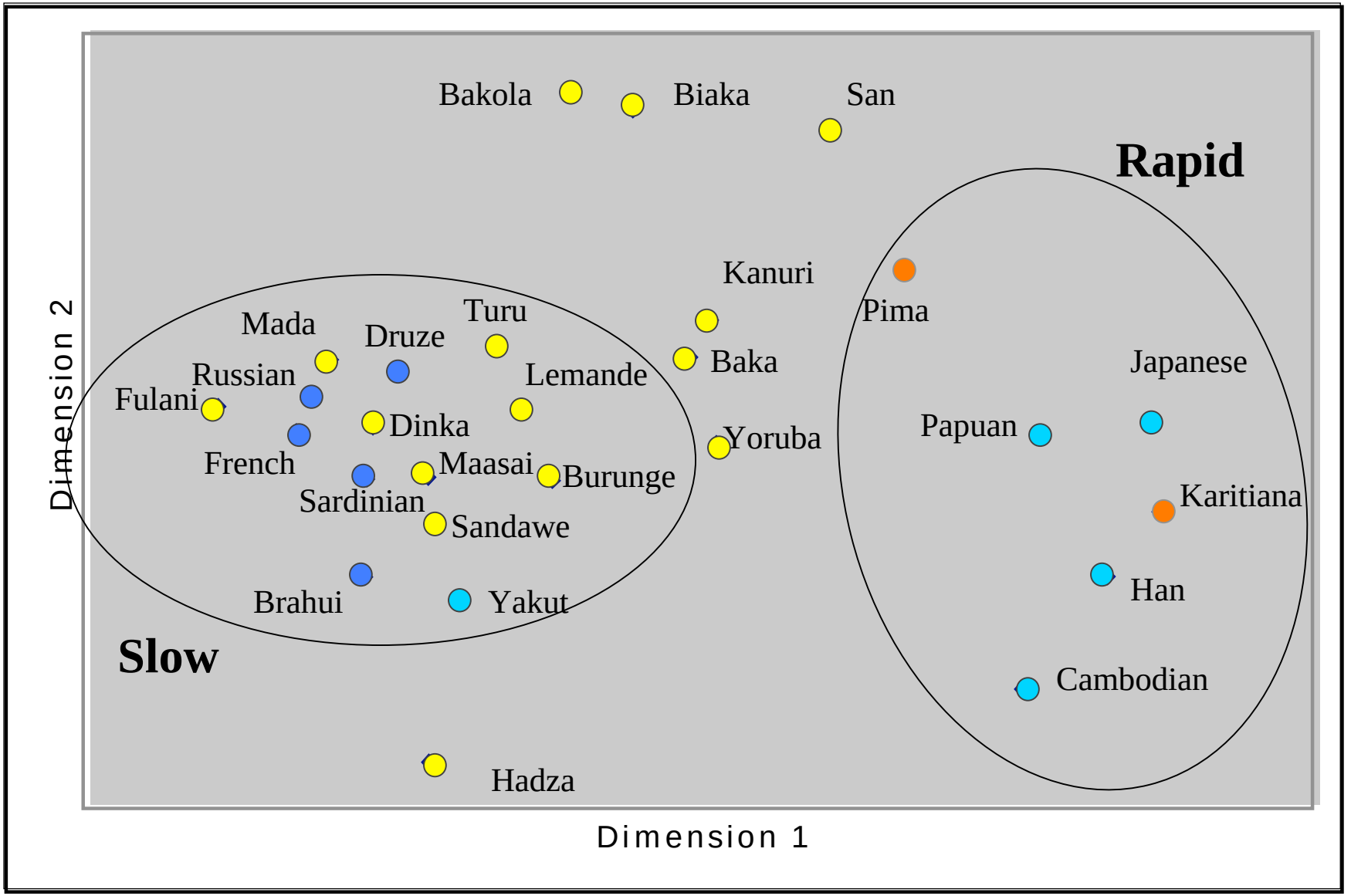


NAT2 Acetylator Haplotype Network-Coding Region

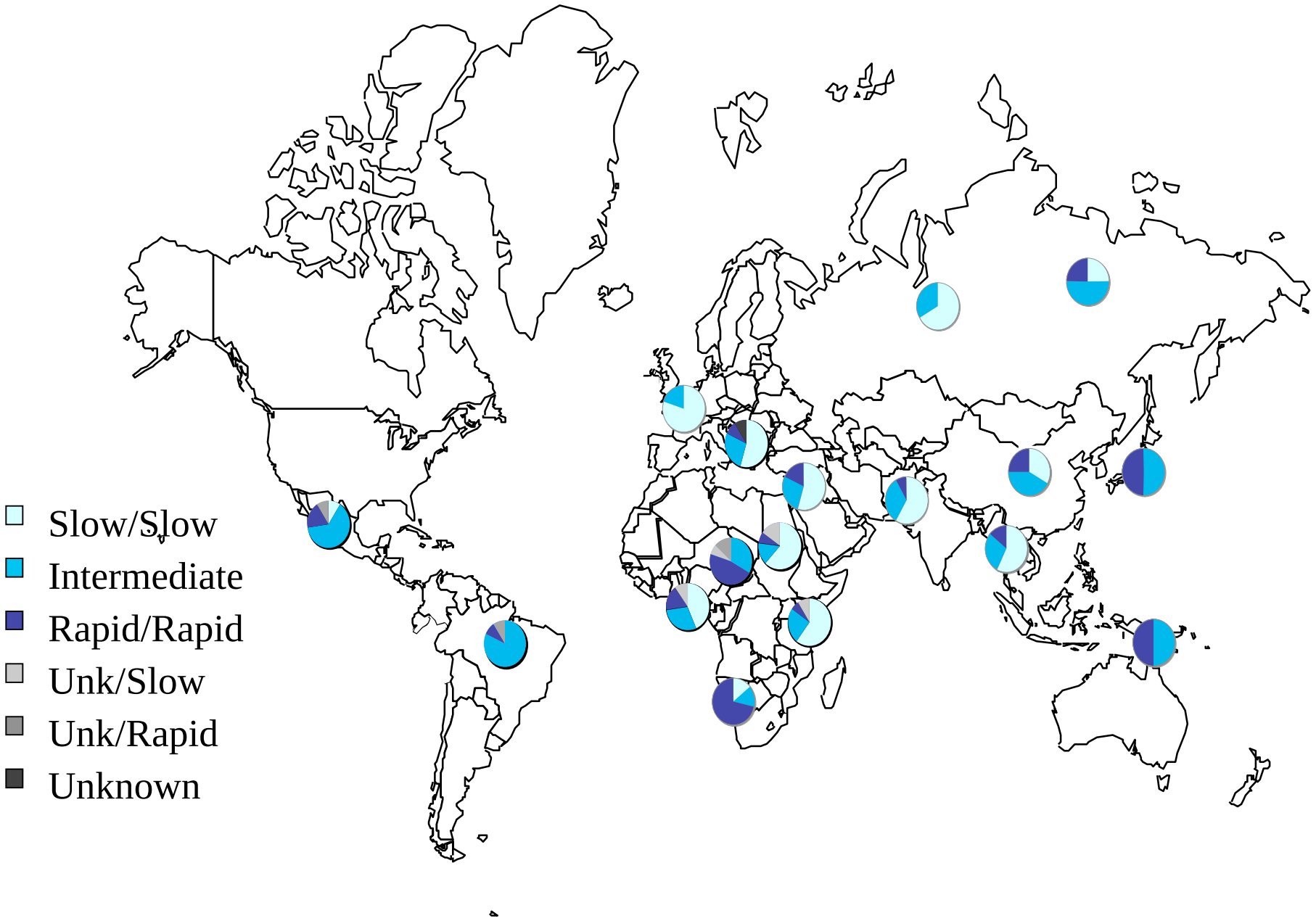


Network 4.5 fluxus-engineering.com (Bandelt *et al.*, 1999)

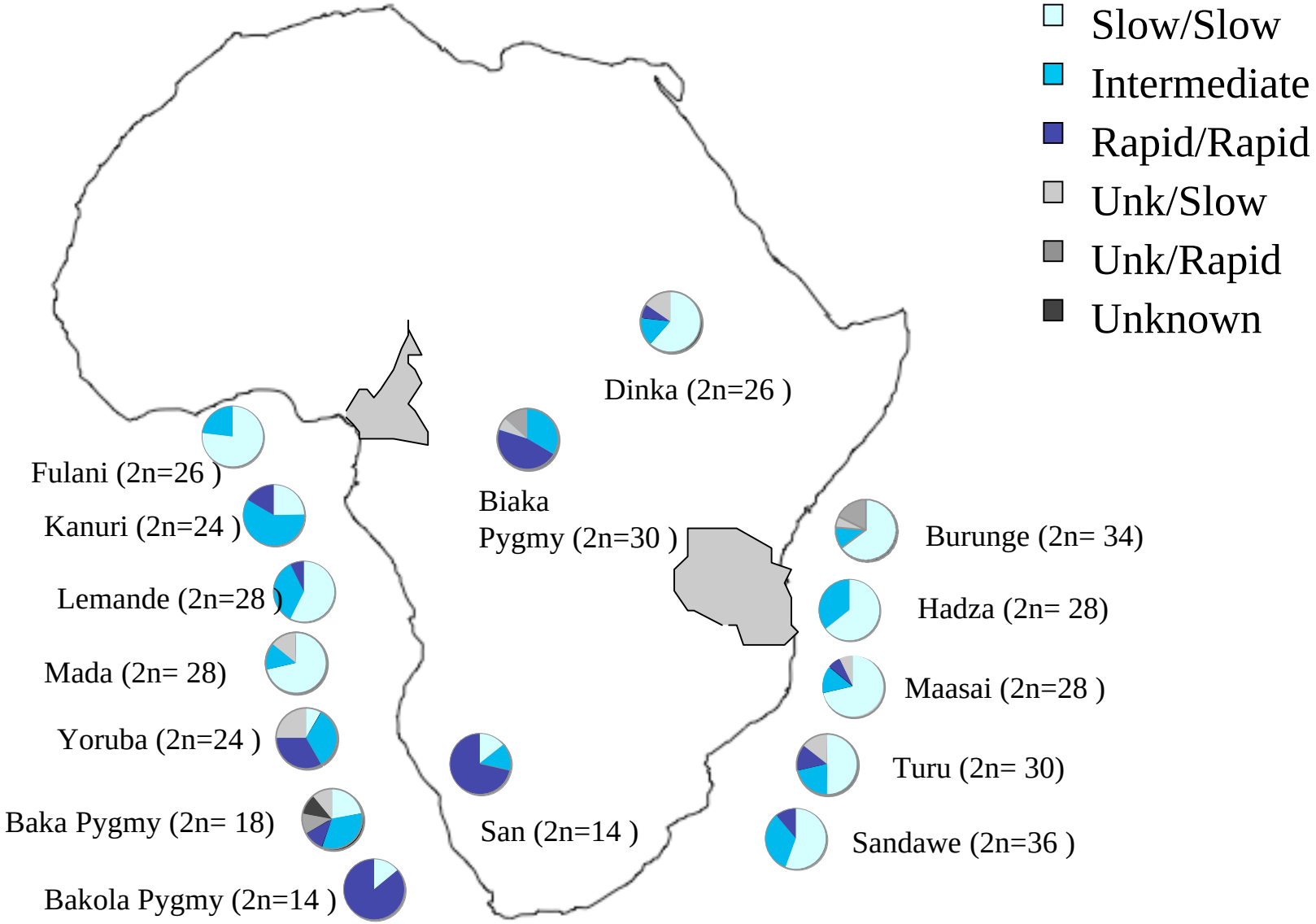
NAT2 Multi-Dimensional Scaling Plot



NAT2 Global Acetylator Frequency Distribution



African NAT2 inferred Acetylation Phenotype



Sequence-Based Neutrality Tests of *NAT2*

NAT2 Coding and Flanking Regions (3031 bp)

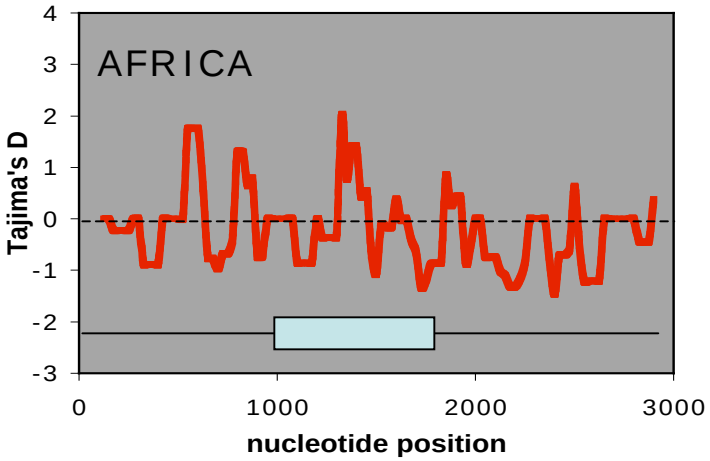
POPULATION	TD	D*	F*	H
Africa	-0.556	-0.647	-0.730	0.822
E Africa	-0.382	-0.705	-0.683	0.714
W Africa	0.023	-2.112	-1.536	0.013
Hadza	-0.179	-0.204	-0.230	-2.392
Pygmy groups	0.014	0.347	0.270	0.167
S. African San	-0.400	-0.397	-0.455	-2.066
Europe	0.797	0.493	0.724	0.323
Asia	0.586	1.437	1.358	0.876
Americas	0.717	1.406	1.398	-0.529

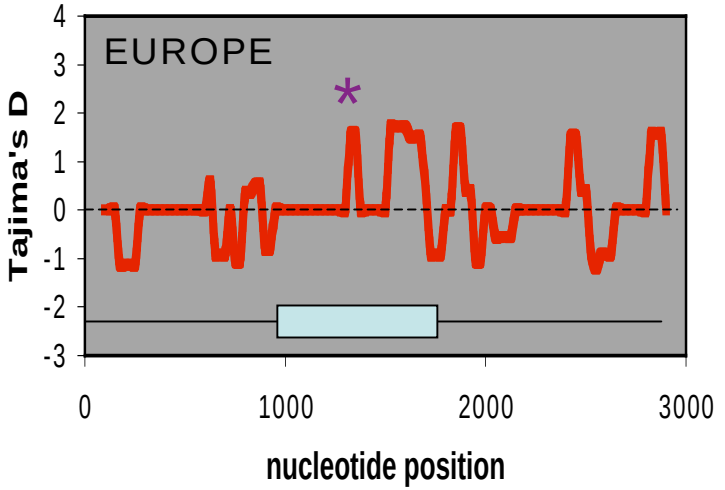
$p<0.05$

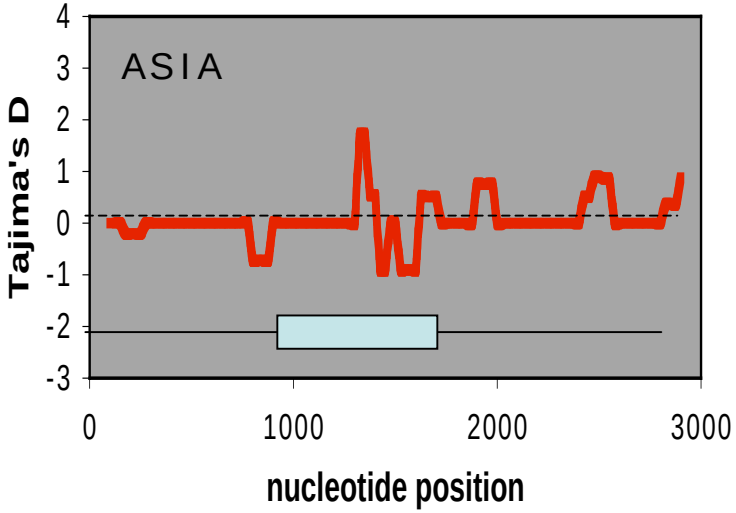
TD (Tajima,1989)
F*/D* (Fu and Li, 1993)
H (Fay and Wu, 2000)

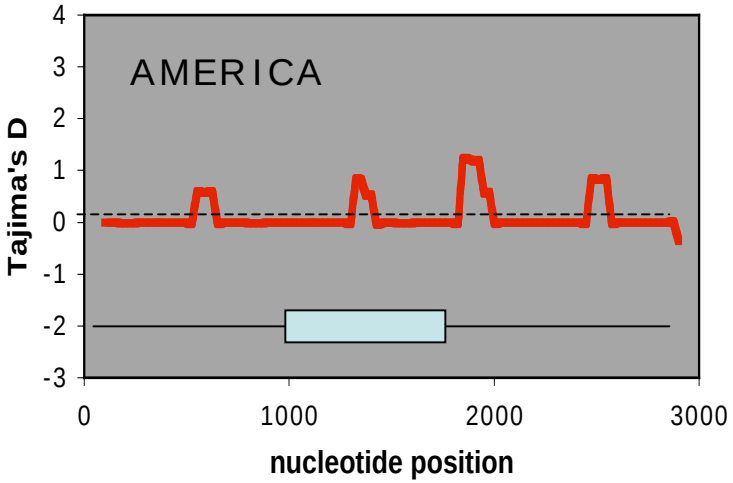
DNAsp 4.20.2 (Rozas and Rozas, 1995)

NAT2 Sliding Window Tajima D



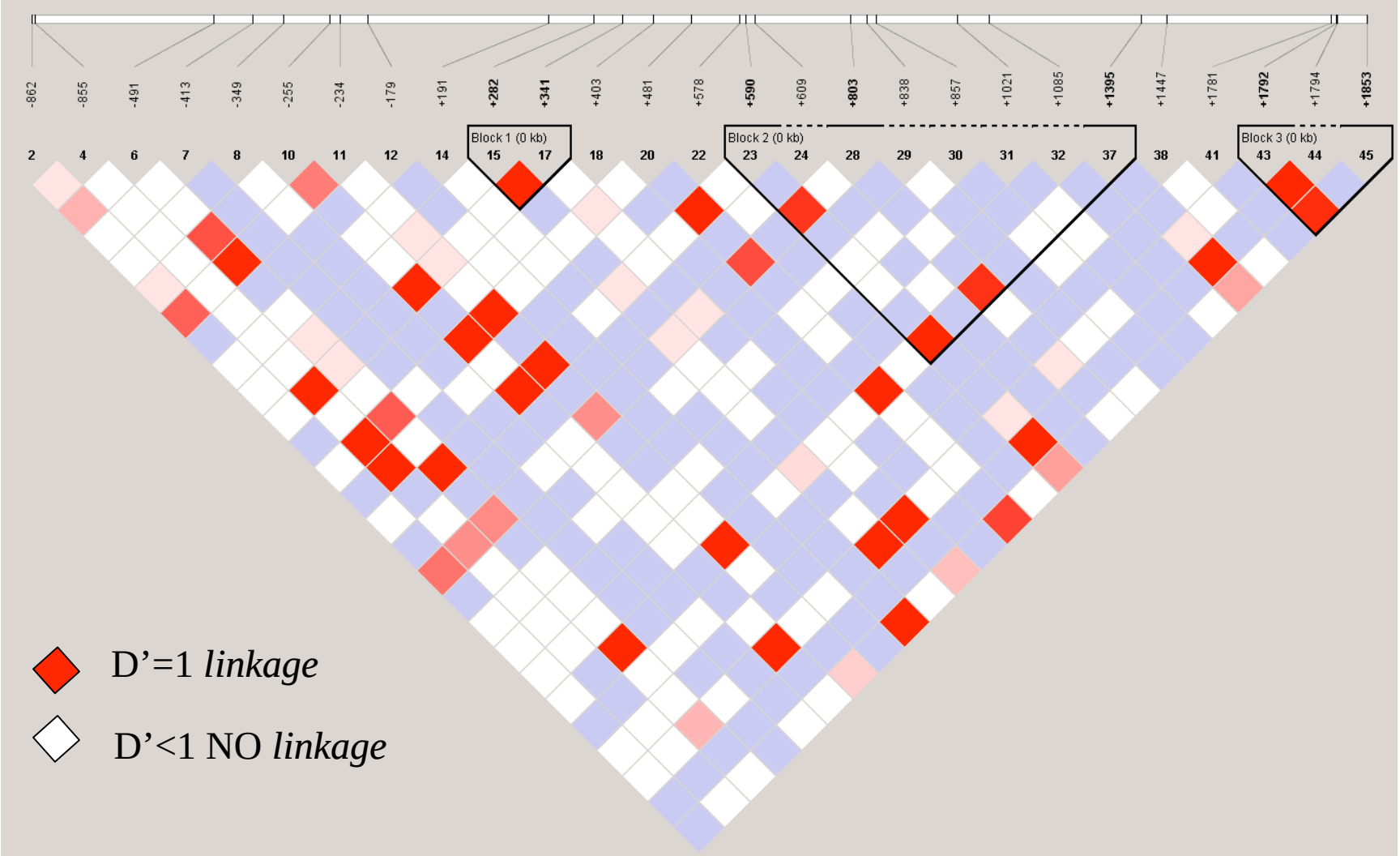






* P<0.05

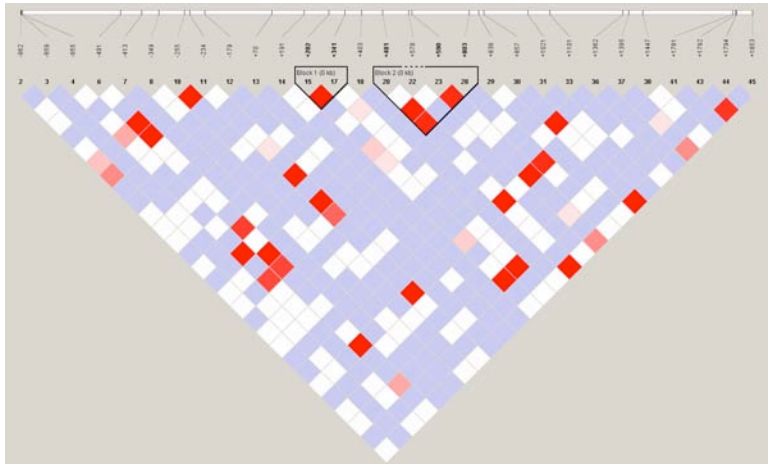
NAT2 Africa: Linkage Disequilibrium (LD)/Recombination Plot



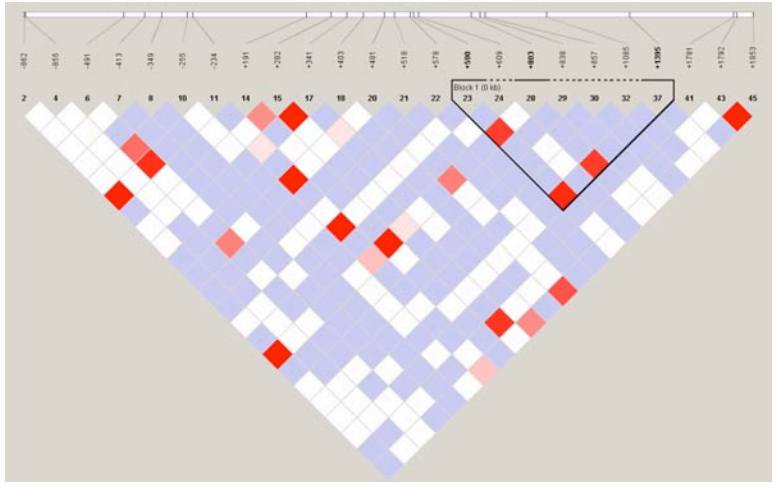
MAF <0.01 excluded

NAT2

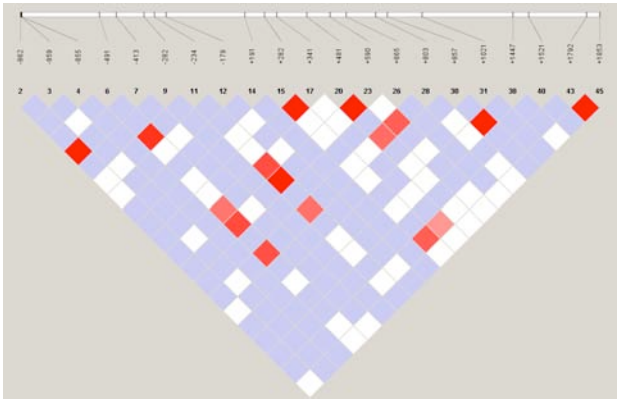
East Africa



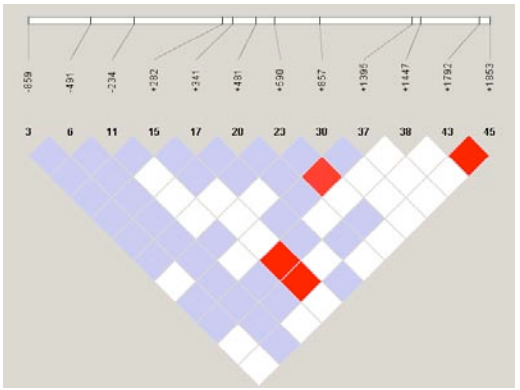
West Africa



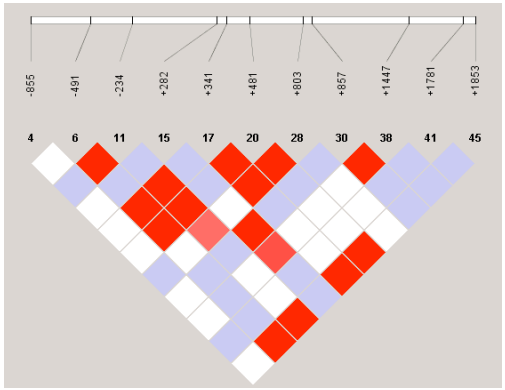
Europe



Asia



Americas



MAF <0.01 excluded

Conclusions

•The *NAT1* data is consistent with two different patterns of selection:

-Purifying selection in the coding region only

-Balancing (or directional) selection affecting the 3' UTR

*Differential polyadenylation may be one way NAT1 phenotype is altered

•The *NAT2* data is consistent with a signature of balancing selection in humans

-*NAT2* data indicate a more complex model of selection

*Different selective forces may be operating in a "population specific" manner

•Further and ongoing investigation will clarify the role these loci have had in past human adaptation and present-day variability in disease risk and drug metabolism

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