NCI-IARC Tumor Workshop: Esophageal Squamous Cell Carcinoma

Genetic and Genomic Research in Africa

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African ESCC: genetics and genomics

1. Genetic association studies to identify germline genetic variants which contribute to the risk of African ESCC
2. DNA analysis of tumour tissue to identify somatic mutations that are drivers of tumorigenesis

- Brief review of published work
- Summarise recent unpublished work
- New funded studies
- Unanswered questions

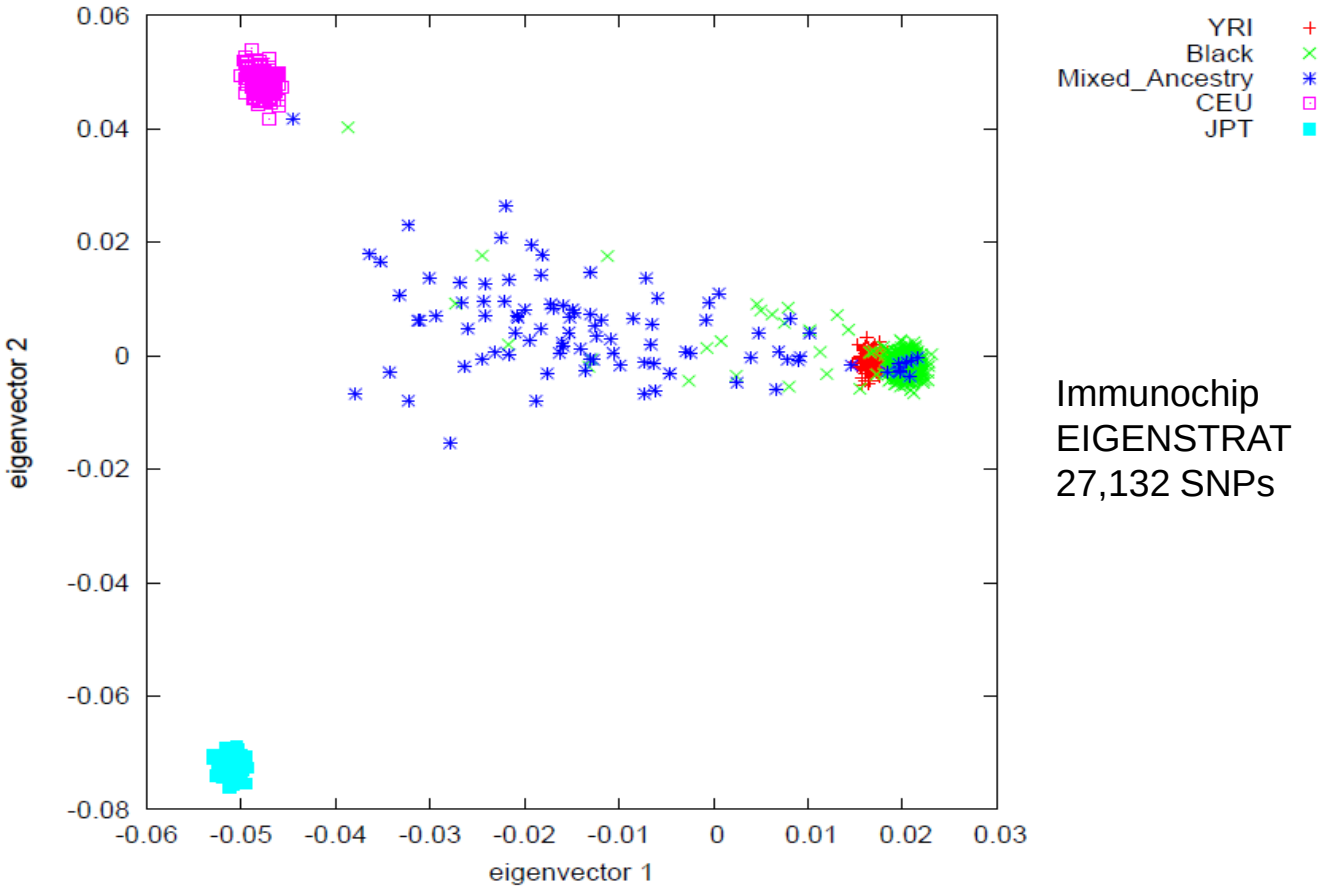
Published genetic associations for ESCC in South Africa

Gene	Association	Authors
Cytochrome P450	CYP2E1*6 increased risk (OR 5.9, P<0.001) CYP3A5*3 reduced risk in MxA (OR 0.60, P=0.025)	Li et al, 2005 Dandara et al, 2005
SLC11A1	-237C>T increased risk in MxA (P<0.002)	Zaahl et al, 2005
SULT1A1	*SULT1A1* R213H increased risk (smokers, OR 2.1)	Dandara et al, 2006
ADH3	Ile350Val increased risk, SAB (OR 1.8, P<0.0004)	Li et al, 2008
ALDH2	+82G>A reduced risk in MxA (OR 1.43, P<0.004)	Bye et al, 2011
GSTP1 *GSTT2B*	341C>T (A114V) increased risk in SAB/MxA (OR 4.98) Deletion reduced risk in MxA (OR 0.71, P=0.004)	Li et al, 2010 Matejcic et al, 2011
Ceruloplasmin	G633G increased risk in SAB (P=0.0004)	Strickland, 2012
MSH3	Ala1045Thr increased risk in MxA (OR 2.71, P=0.006)	Vogelsang et al, 2012
PLCE1	rs2274223 not associated in SAB (OR 1.06, P=0.52) Arg548Leu reduced risk in SAB (OR 0.74, P=0.008)	Bye et al, 2012
NAT2	rs1801280 reduced risk in MxA (OR 0.31, P=0.026)	Matejcic et al, 2015

SAB: South African Black population
MxA: Mixed Ancestry population, Western Cape

Population structure

- >2000 linguistic/ethnic groups in Africa, with differences in genetic architecture
- Need to genotype a large number of SNPs to correct for population structure
- Principal component analysis shows tight clustering of Xhosa-speakers, but heterogeneity in the Cape Mixed Ancestry population



Immunochip
EIGENSTRAT
27,132 SNPs

ESCC GWAS loci in the South African Black population

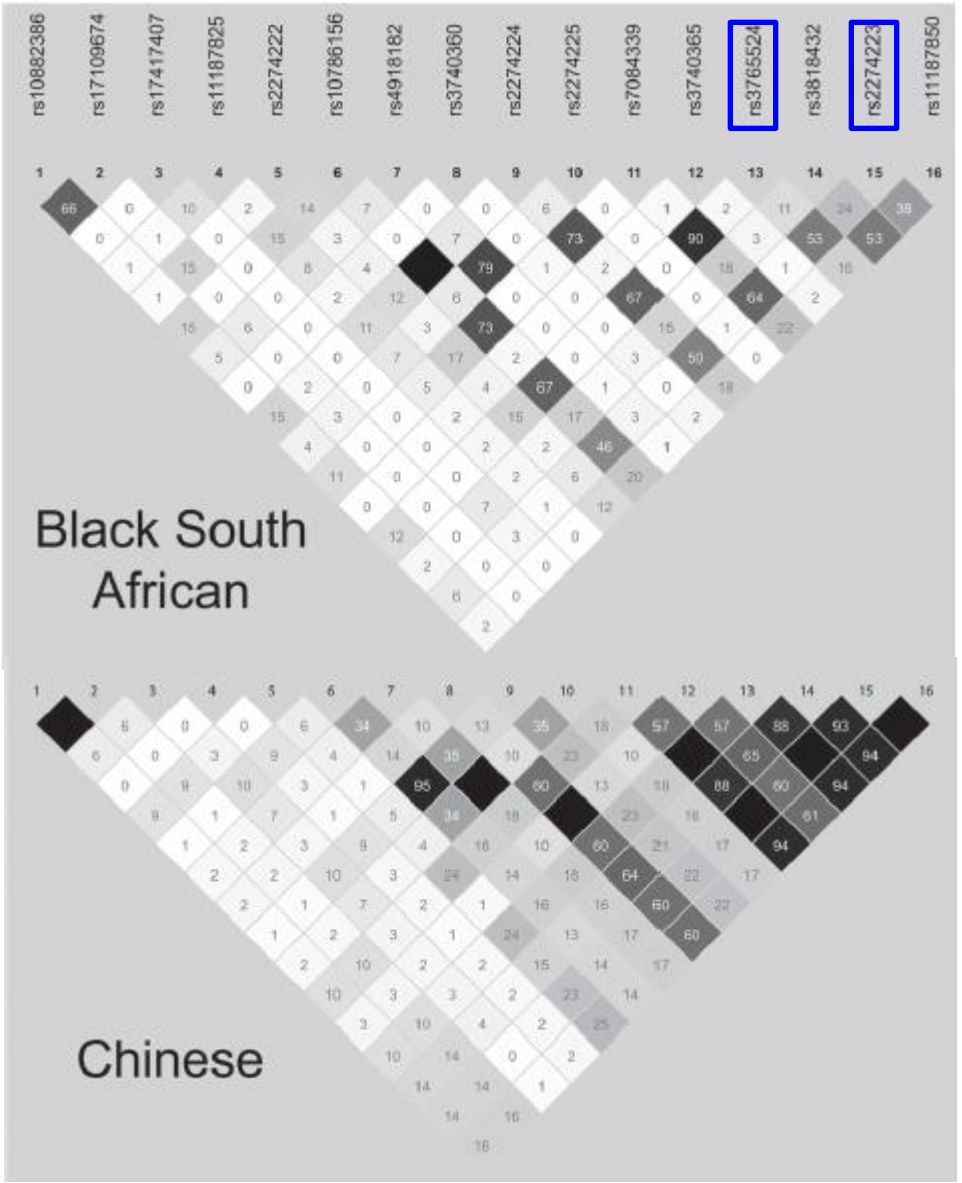
Locus	SNP	MAF Cases	MAF Controls	OR (95% CI)	P value
RUNX1/2q22	rs2014300	0.378	0.403	0.90 (0.76-1.07)	0.23
CASP8/2q33	rs13016963	0.330	0.340	0.96 (0.79-1.17)	0.68
TMEM173/5q31	rs13153461*	0.046	0.047	0.98 (0.68-1.42)	1
PLCE1/10q23	rs2274223	0.416	0.403	1.06 (0.89-1.25)	0.52
ATP1B2/TP53/17p	rs1642764	0.230	0.194	1.24* (1.02-1.52)	0.031
XBP1/22q12	rs2239815	0.22	0.16	1.41 (1.15 – 1.73)	0.0008
CHEK2/22q12	rs4822983	0.46	0.39	1.31 (1.11 – 1.54)	0.0013
CHEK2/22q12	rs1033667	0.44	0.38	1.29 (1.09 – 1.51)	0.0025

Genotyping in 513 ESCC SAB cases and 820 controls
ADH1B Arg48His and *ALDH2* Glu504Lys are not polymorphic in the SAB population

*Proxy for rs7447927 – failed assay design
*Opposite effect to Chinese GWAS

Linkage disequilibrium at *PLCE1* in South African vs Chinese populations

- Much lower LD in Black South African population
- Dense SNP map required to detect association
- Advantage for fine-mapping causal variants



New and proposed African ESCC association studies

- Agena MassArray genotyping (36 SNP-plexes)) of known ESCC loci and ancestry informative markers in 1000 Black SA cases and 1000 controls from Johannesburg Cancer Study (JCS) – replication and fine-mapping (CANSA/U.Wits)
- GWAS of 2000 SAB ESCC cases (JCS & Western Cape) and 6000 controls (Johannesburg/Soweto H3A/NIH) using H3A pan-African GWAS SNP array* (Newton fund: SA/UK MRC, GSK)
- IARC/NCI case-control studies in East Africa (Van Loon, McCormack et al)
- Planned project grant application to NIH H3 Africa call: additional ESCC recruitment/GWAS in South & East Africa

*Illumina array: 2.5M SNPs incl. MEGA content; 750K tag SNPs selected from WGS of 3500 African genomes; previous GWAS hits; majority SNPs MAF >5%

Somatic mutations in African OSSC

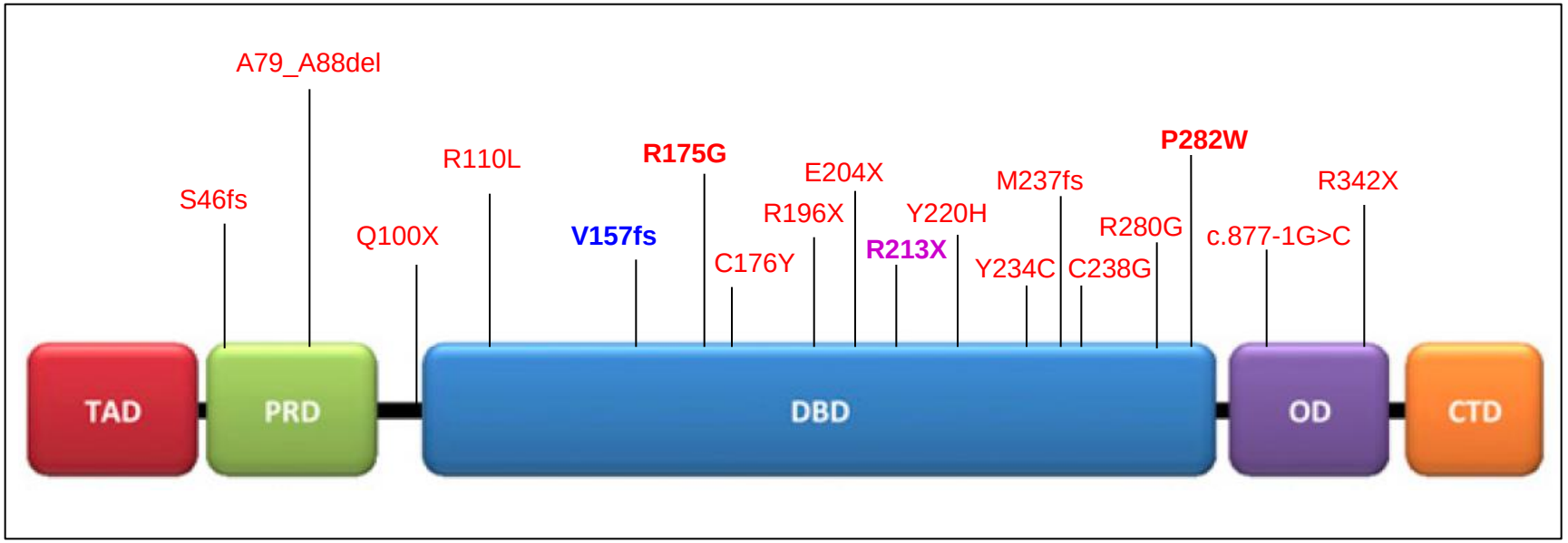
Main approaches used to screen tumours:

- Comparative genomic hybridisation, FISH or LOH analysis to screen for deletion or amplification of genomic regions
- Screening of candidate genes (mainly *TP53*) to detect point mutations in tumour with/without paired normal tissue

Authors	Methods	Findings
Gameldien et al, 1998	SSCP of *TP53* exons 5-8 & *CDKN2A* exons 1-2, sequencing	From 76 SCCs, Transkei: *TP53* mutations in 17%; *CDKN2A* in 21%
Du Plessis et al, 1999	CGH of 27 OSCCs & 2 AOCs, SAB & MxA	Freq loss (>50%) chr 1p, 4p, 19p/q Freq gain (>50%) chr 3q, Xq, 8q, 2q
Naidoo et al, 2005	Microsatellite repeat instability & LOH, 6 MS in 100 OSCCs/KZN	MSI very low: 0-5% LOH was 18-41%
Brown et al, 2011	Cytogenetics, M-FISH, SNP array 5 SA OSCC cell lines	Common translocn 1p11-12/3p11.2 Gains/losses > Wnt & FGF signalling
Patel et al, 2011	28 OSCCs from Kenya Sequencing exons 5-8 *TP53*	*TP53* mutations detected in 39% All negative for 19 HPV types

African ESCC somatic mutations: unpublished/new studies

- Pilot whole exome sequencing of 10 SAB blood/tumour pairs
- Recurrently mutated:*TP53* (7), *ATR* (2), *GNAS* (2), *MAGI2* (2)
- Other mutations: *FBXW7, FLT3, NFE2L2, TET2, ZNF750*
- Sanger sequencing:*TP53* mutated in 18/26 tumours (69%)



- Newton fund: Parker, Mathew, Campbell et al: Whole genome sequencing of 30 ESCC (SAB) B/T pairs & RNA-seq > driver mutations and mutational signatures. Follow up sequencing of gene panels in ~300 ESCC

Summary and Conclusions

- Few robust genetic associations with African OSCC thus far; *XBP1/CHEK2* locus looks promising
- Lack of replication may reflect
  - modest sample sizes, statistical power,
  - lower linkage disequilibrium with causal variants in African populations would also reduce power
  - population-specific differences owing to absence of causal variants and different gene x environmental interactions

- Limited genomic screens for somatic mutations thus far
- *TP53* mutations common in South African and Kenyan OSCC

African ESCC Genetics/Genomics: Unanswered Questions

- How much does genetic variation contribute to the development of African ESCC, and are their regional differences?
- Shorter blocks of linkage disequilibrium in African populations implies denser genotyping panels; WGS may be needed for full discovery
- Need substantial additional case/control recruitment for well powered GWAS, a collaborative approach for replication studies/meta-analysis and $$$
- What are the major genomic drivers of tumorigenesis in African ESCC? Need to do WGS on substantial numbers of blood/tumour pairs (ICGC standard is 500)
- Will extraction of mutational signatures from WGS of tumours provide useful insights into environmental risk factors?