Viral and genomic drivers of epithelial neoplasms arising in the lacrimal drainage system

Supplementary Material

Supplementary Legends

Supplementary Table 1: The 523 genes included in the TruSight Oncology (TSO500) Gene Panel (Illumina, San Diego, CA, USA).
Supplementary Table 2: Ingenuity Variant Analysis filtering criteria (based on the TSO500 default filtering)
Supplementary Table 3: All annotated variants.
Supplementary Table 4: Studies reporting on Human papillomavirus (HPV) in inverted papillomas of the lacrimal drainage system.
Supplementary Figure 1: The PI3K-signalling cascade, simplified. Created with BioRender.com.

Supplementary Tables

Supplementary Table 1

ABL1	CDKN2C	FAS	HOXB13	MRE11A	PPARG	SOX2
ABL2	CEBPA	FAT1	HRAS	MSH2	PPM1D	SOX9
ACVR1	CENPA	FBXW7	HSD3B1	MSH3	PPP2R1A	SPEN
ACVR1B	CHD2	FGF1	HSP90AA1	MSH6	PPP2R2A	SPOP
AKT1	CHD4	FGF10	ICOSLG	MST1	PPP6C	SPTA1
AKT2	CHEK1	FGF14	ID3	MST1R	PRDM1	SRC
AKT3	CHEK2	FGF19	IDH1	MTOR	PREX2	SRSF2
ALK	CIC	FGF2	IDH2	MUTYH	PRKAR1A	STAG1
ALOX12B	CREBBP	FGF23	IFNGR1	MYB	PRKCI	STAG2
ANKRD11	CRKL	FGF3	IGF1	MYC	PRKDC	STAT3
ANKRD26	CRLF2	FGF4	IGF1R	MYCL1	PRSS8	STAT4
APC	CSF1R	FGF5	IGF2	MYCN	PTCH1	STAT5A
AR	CSF3R	FGF6	IKBKE	MYD88	PTEN	STAT5B

ARAF	CSNK1A1	FGF7	IKZF1	MYOD1	PTPN11	STK11
ARFRP1	CTCF	FGF8	IL10	NAB2	PTPRD	STK40
ARID1A	CTLA4	FGF9	IL7R	NBN	PTPRS	SUFU
ARID1B	CTNNA1	FGFR1	INHA	NCOA3	PTPRT	SUZ12
ARID2	CTNNB1	FGFR2	INHBA	NCOR1	QKI	SYK
ARID5B	CUL3	FGFR3	INPP4A	NEGR1	RAB35	TAF1
ASXL1	CUX1	FGFR4	INPP4B	NF1	RAC1	TBX3
ASXL2	CXCR4	FH	INSR	NF2	RAD21	TCEB1
ATM	CYLD	FLCN	IRF2	NFE2L2	RAD50	TCF3
ATR	DAXX	FLI1	IRF4	NFKBIA	RAD51	TCF7L2
ATRX	DCUN1D1	FLT1	IRS1	NKX2-1	RAD51B	TERC
AURKA	DDR2	FLT3	IRS2	NKX3-1	RAD51C	TERT
AURKB	DDX41	FLT4	JAK1	NOTCH1	RAD51D	TET1
AXIN1	DHX15	FOXA1	JAK2	NOTCH2	RAD52	TET2
AXIN2	DICER1	FOXL2	JAK3	NOTCH3	RAD54L	TFE3
AXL	DIS3	FOXO1	JUN	NOTCH4	RAF1	TFRC
B2M	DNAJB1	FOXP1	KAT6A	NPM1	RANBP2	TGFBR1
BAP1	DNMT1	FRS2	KDM5A	NRAS	RARA	TGFBR2
BARD1	DNMT3A	FUBP1	KDM5C	NRG1	RASA1	TMEM127
BBC3	DNMT3B	FYN	KDM6A	NSD1	RB1	TMPRSS2
BCL10	DOT1L	GABRA6	KDR	NTRK1	RBM10	TNFAIP3
BCL2	E2F3	GATA1	KEAP1	NTRK2	RECQL4	TNFRSF14
BCL2L1	EED	GATA2	KEL	NTRK3	REL	TOP1
BCL2L11	EGFL7	GATA3	KIF5B	NUP93	RET	TOP2A
BCL2L2	EGFR	GATA4	KIT	NUTM1	RFWD2	TP53
BCL6	EIF1AX	GATA6	KLF4	PAK1	RHEB	TP63
BCOR	EIF4A2	GEN1	KLHL6	PAK3	RHOA	TRAF2
BCORL1	EIF4E	GID4	KMT2B	PAK7	RICTOR	TRAF7
BCR	EML4	GLI1	KMT2C	PALB2	RIT1	TSC1
BIRC3	EP300	GNA11	KMT2D	PARK2	RNF43	TSC2
BLM	EPCAM	GNA13	KRAS	PARP1	ROS1	TSHR
BMPR1A	EPHA3	GNAQ	LAMP1	PAX3	RPS6KA4	U2AF1

BRAF
BRCA1
BRCA2
BRD4
BRIP1
BTG1
BTK
C11orf30
CALR
CARD11
CASP8
CBFB
CBL
CCND1
CCND2
CCND3
CCNE1
CD274
CD276
CD74
CD79A
CD79B
CDC73
CDH1
CDK12
CDK4
CDK6
CDK8
CDKN1A
CDKN1B
CDKN2A
CDKN2B
EPHA5
EPHA7
EPHB1
ERBB2
ERBB3
ERBB4
ERCC1
ERCC2
ERCC3
ERCC4
ERCC5
ERG
ERRFI1
ESR1
ETS1
ETV1
ETV4
ETV5
ETV6
EWSR1
EZH2
FAM123B
FAM175A
FAM46C
FANCA
FANCC
FANCD2
FANCE
FANCF
FANCG
FANCI
FANCL
GNAS
GPR124
GPS2
GREM1
GRIN2A
GRM3
GSK3B
H3F3A
H3F3B
H3F3C
HGF
HIST1H1C
HIST1H2BD
HIST1H3A
HIST1H3B
HIST1H3C
HIST1H3D
HIST1H3E
HIST1H3F
HIST1H3G
HIST1H3H
HIST1H3I
HIST1H3J
HIST2H3A
HIST2H3C
HIST2H3D
HIST3H3
HLA-A
HLA-B
HLA-C
HNF1A
HNRNPK
LATS1
LATS2
LMO1
LRP1B
LYN
LZTR1
MAGI2
MALT1
MAP2K1
MAP2K2
MAP2K4
MAP3K1
MAP3K13
MAP3K14
MAP3K4
MAPK1
MAPK3
MAX
MCL1
MDC1
MDM2
MDM4
MED12
MEF2B
MEN1
MET
MGA
MITF
MLH1
MLL
MLLT3
MPL
PAX5
PAX7
PAX8
PBRM1
PDCD1
PDCD1LG2
PDGFRA
PDGFRB
PDK1
PDPK1
PGR
PHF6
PHOX2B
PIK3C2B
PIK3C2G
PIK3C3
PIK3CA
PIK3CB
PIK3CD
PIK3CG
PIK3R1
PIK3R2
PIK3R3
PIM1
PLCG2
PLK2
PMAIP1
PMS1
PMS2
PNRC1
POLD1
POLE
RPS6KB1
RPS6KB2
RPTOR
RUNX1
RUNX1T1
RYBP
SDHA
SDHAF2
SDHB
SDHC
SDHD
SETBP1
SETD2
SF3B1
SH2B3
SH2D1A
SHQ1
SLIT2
SLX4
SMAD2
SMAD3
SMAD4
SMARCA4
SMARCB1
SMARCD1
SMC1A
SMC3
SMO
SNCAIP
SOCS1
SOX10
SOX17
VEGFA
VHL
VTCN1
WISP3
WT1
XIAP
XPO1
XRCC2
YAP1
YES1
ZBTB2
ZBTB7A
ZFHX3
ZNF217
ZNF703
ZRSR2

Supplementary Table 2

Filter	Inclusion criteria
Confidence	Call quality > 20
	Variant passed upstream pipeline filtering
	Read depth > 100
	Allele fraction > 10
	Outside top 5% most exonically variable 100base windows in healthy public genomes
	Outside top 1% most exonically variable genes in healthy public genomes (100Genomes)
Common variants	<1% of all in gnomAD
	<1% of all in ExAc
	<1% of all in NHLBI ESP exomes
	<1% of all in the 1000 Genomes Project
Predicted deleterious	Pathogenic variants (ACMG Guidelines classification)
	Likely pathogenic variants (ACMG Guidelines classification)
	Uncertain significance (ACMG Guidelines classification)
	Variants listed in HGMD, ClinVar, CentoMD
	Variants established in the literature

Supplementary Table 3

Sample	HPV DNA	HPV E6/E7	p16	HPV genotype	Diagnosis	Read depth	Chromosome location	Gene	AA Change	Transcript	Nucleotide change	Translational impact	Variant allele	Computed classification*
1	Positive	Positive	Positive	HPV11	NKSCC	229	chr3:30713499	*TGFBR2*	p.A275E	NM_003242.6	c.824C>A	Gain	61	Uncertain
						181	chr22:23653975	*BCR*	p.V1050fs*17	NM_021574.3	c.3143_3146dupCC	Loss	14	Uncertain
2	Positive	Positive	Negative	HPV11	NKSCC	238	chrX:39913571	*BCOR*	NA	NM_017745.6	c.4640-105_4655delTCCAGCCTGTCATGAATGTATAGGAAAAAATGCCACGTGGCTCTGCCATCATTTTGTTCTCAGCAGTAGATTTTATCTTGTTTTTTTATTTTTCTCTCCCTCCAGATTATTTAAATGACCT	Loss	18	Likely pathogenic
						318	chr17:7576855	*TP53*	p.Q331*	NM_000546.6	c.991C>T	Loss	30	Pathogenic
						231	chr9:139401863	*NOTCH1*	p.N1179fs*4	NM_017617.5	c.3536dupA	Loss	26	Uncertain
						353	chrX:44929416	*KDM6A*	p.N839fs*6	NM_021140.4	c.2517_2518delCA	Loss	24	Likely pathogenic
						236	chr22:23653975	*BCR*	p.V1050fs*17	NM_021574.3	c.3143_3146dupCCGG	Loss	10	Uncertain
3	Positive	Positive	Negative	HPV11	NKSCC	218	chr1:120462032	*NOTCH2*	p.R1895H	NM_024408.4	c.5684G>A	NA	47	Uncertain
						306	chr3:30713506	*TGFBR2*	p.K277N	NM_003242.6	c.831G>T	Gain	63	Uncertain
						147	chr7:106526601	*PIK3CG*	p.F965S	NM_002649.3	c.2894T>C	Loss	53	Uncertain
						214	chr17:47696716	*SPOP*	p.E78K	NM_003563.3	c.232G>A	Loss	26	Likely pathogenic
4	Negative	Negative	Negative	NA	NKSCC	454	chr12:49448470	*KMT2D*	p.E81*	NM_003482.4	c.241G>T	Loss	36	Likely pathogenic
						417	chr12:49444974	*KMT2D*	p.S831*	NM_003482.4	c.2492C>G	Loss	33	Likely pathogenic
						349	chr17:7579579	*TP53*	p.S37fs*7	NM_000546.6	c.108delG	Frameshift	55	Likely pathogenic
5	Positive	Positive	Positive	HPV16	SCC	101	chr3:30691871	*TGFBR2*	p.P129fs*3	NM_003242.6	c.383dupA	Loss	10	Uncertain
						314	chr11:22647208	*FANCF*	p.R50L	NM_022725.4	c.149G>T	NA	25	Uncertain
						1071	chr15:90631914	*IDH2*	p.V147I	NM_002168.4	c.439G>A	NA	61	Uncertain
						654	chr22:23653975	*BCR*	p.V1050fs*17	NM_021574.3	c.3143_3146dupCCGG	Loss	15	Uncertain
6	Positive	Positive	Positive	HPV16	SCC	902	chr5:67591211	*PIK3R1*	NA	NM_181523.3	c.1746-35_1748delAGTTAATGCGTTCTCTTTTCAAAACTGTTTTTCAGGTG	Loss	17	Likely pathogenic
						361	chr16:72822574	*ZFHX3*	p.Q3201*	NM_001164766.2	c.9601C>T	Loss	40	Likely pathogenic

Sample	HPV DNA	HPV E6/E7	p16	HPV genotype	Diagnosis	Read depth	Chromosome location	Gene	AA Change	Transcript	Nucleotide change	Translational impact	Variant allele	Computed classification*
7	Positive	Positive	Positive	HPV16	SCC	140	chr3:178936082	*PIK3CA*	p.E542K	NM_006218.4	c.1624G>A	Gain	36	Pathogenic
8	Positive	Positive	Positive	HPV16	SCC	174	chr3:178936091	*PIK3CA*	p.E545K	NM_006218.4	c.1633G>A	Gain	40	Pathogenic
						131	chr13:49027168	*RB1*	p.R579*	NM_000321.3	c.1735C>T	Loss	21	Pathogenic
						102	chr16:3781324	*CREBBP*	p.S1642del	NM_001079846.1	c.4925_4927delCCT	Loss	25	Pathogenic
						112	chr11:108206607	*ATM*	p.Q2729H	NM_000051.4	c.8187A>T	Loss	21	Likely pathogenic
						142	chr1:16259147	*SPEN*	p.S2139*	NM_015001.3	c.6414_6415delAA	Loss	24	Likely pathogenic
						139	chr1:16257629	*SPEN*	p.K1632fs*28	NM_015001.3	c.4896_4897delAA	Loss	20	Likely pathogenic
9	Positive	Positive	Positive	HPV16	SCC	127	chr3:178916957	*PIK3CA*	p.R115L	NM_006218.4	c.344G>T	Gain	17	Pathogenic
						113	chr5:67593270	*PIK3R1*	p.N673fs*18	NM_181523.3	c.2019_2022delCAAA	Loss	38	Likely pathogenic
10	Positive	Positive	Positive	HPV16	SCC	273	chr4:1803568	*FGFR3*	p.S249C	NM_000142.5	c.746C>G	Gain	37	Pathogenic
						141	chr1:27023696	*ARID1A*	p.Q268*	NM_006015.6	c.802C>T	Loss	72	Likely pathogenic
						619	chr12:46231185	*ARID2*	p.L370*	NM_152641.4	c.1109delT	Loss	33	Likely pathogenic
11	Positive	Positive	Positive	HPV16	SCC	971	chr14:105246551	*AKT1*	p.E17K	NM_005163.2	c.49G>A	Gain	42	Pathogenic
						493	chr4:1803568	*FGFR3*	p.S249C	NM_000142.5	c.746C>G	Gain	12	Pathogenic
						1142	chr5:112175211	*APC*	p.I1307K	NM_000038.6	c.3920T>A	Loss	50	Pathogenic
12**	Negative	Negative	Negative	NA	SCC	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	Negative	Negative	Negative	NA	Adenocarcin	531	chr17:29677208	*NF1*	p.T2423fs*4	NM_000267.3	c.7267dupA	Loss	28	Likely pathogenic
						631	chr17:7577085	*TP53*	p.E285K	NM_000546.6	c.853G>A	Loss	26	Pathogenic

* Computed classification of pathogenicity by Qiagen Interpret, USA, based on: Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-24. doi: 10.1038/gim.2015.30. Epub 2015 Mar 5. PMID: 25741868; PMCID: PMC4544753.

Study	N (cases)	Median age (range)	Gender	HPV positive (%)	HPV genotype	HPV detection modality	Recurrence	Malignant transformation
Wang *et al.* 2021[1]	13	57 (43-84)	7M / 6F	2 / 9 (22)	HPV16, 18, 31, 33, 51	Unknown	2 / 13, median follow-up 48 months (range 2 - 241)	4 / 13 (44)
Jones *et al.* 2020[2]	2	NA	NA	2 / 2 (100)	11, 16	HPV DNA PCR	NA	NA
Raemdock *et al.* 2009[3]	1	24	F	1 / 1	Low-risk	HPV DNA ISH	0 / 1 (follow-up 2 years)	0 / 1
Present study	5	52 (45-56)	2M / 3F	3 / 4 (75)	HPV11, 16	HPV DNA PCR, p16, E6/E7 mRNA ISH	2 / 6, median follow up time 96 months (range 2 - 176)	2 / 6 (33)
Total	**22**	**55 (24-84)**	**10M / 10F**	**8 / 16 (50)**	**Low-risk: HPV11 High-risk: HPV16,18, 31, 33, 51**		**4 / 20, median follow-up 48 months (range 2-241)**	**6 / 20 (32)**

Supplementary Table 4

1. J. Wang, J. Ford, B. Esmaeli, P. Langer, N. Esmaili, G. J. Griepentrog, et al. *Inverted papilloma of the orbit and nasolacrimal system.* Ophthalmic Plast Reconstr Surg 2021 Vol. 37 Issue 2 Pages 161-167. DOI: 10.1097/iop.0000000000001719.

2. H. Jones, S. Gane, J. Rimmer, K. Cuschieri and V. J. Lund. *HPV may not play a role in all lacrimal transitional cell papilloma.* Rhinology 2020 Vol. 10.4193/Rhin19.283. DOI: 10.4193/Rhin19.283

3. T. Y. Raemdonck, C. M. Van den Broecke, I. Claerhout and C. E. Decock. *Inverted papilloma arising primarily from the lacrimal sac.* Orbit 2009 Vol. 28 Issue 2-3 Pages 181-4. DOI: 10.1080/01676830802692914

Supplementary Figures

Supplementary Figure 1