GENETICS OF ALCOHOL-RELATED ORAL CARCINOMA: ISSUES WITH ALCOHOL CONTAINING MOUTHWASHES.

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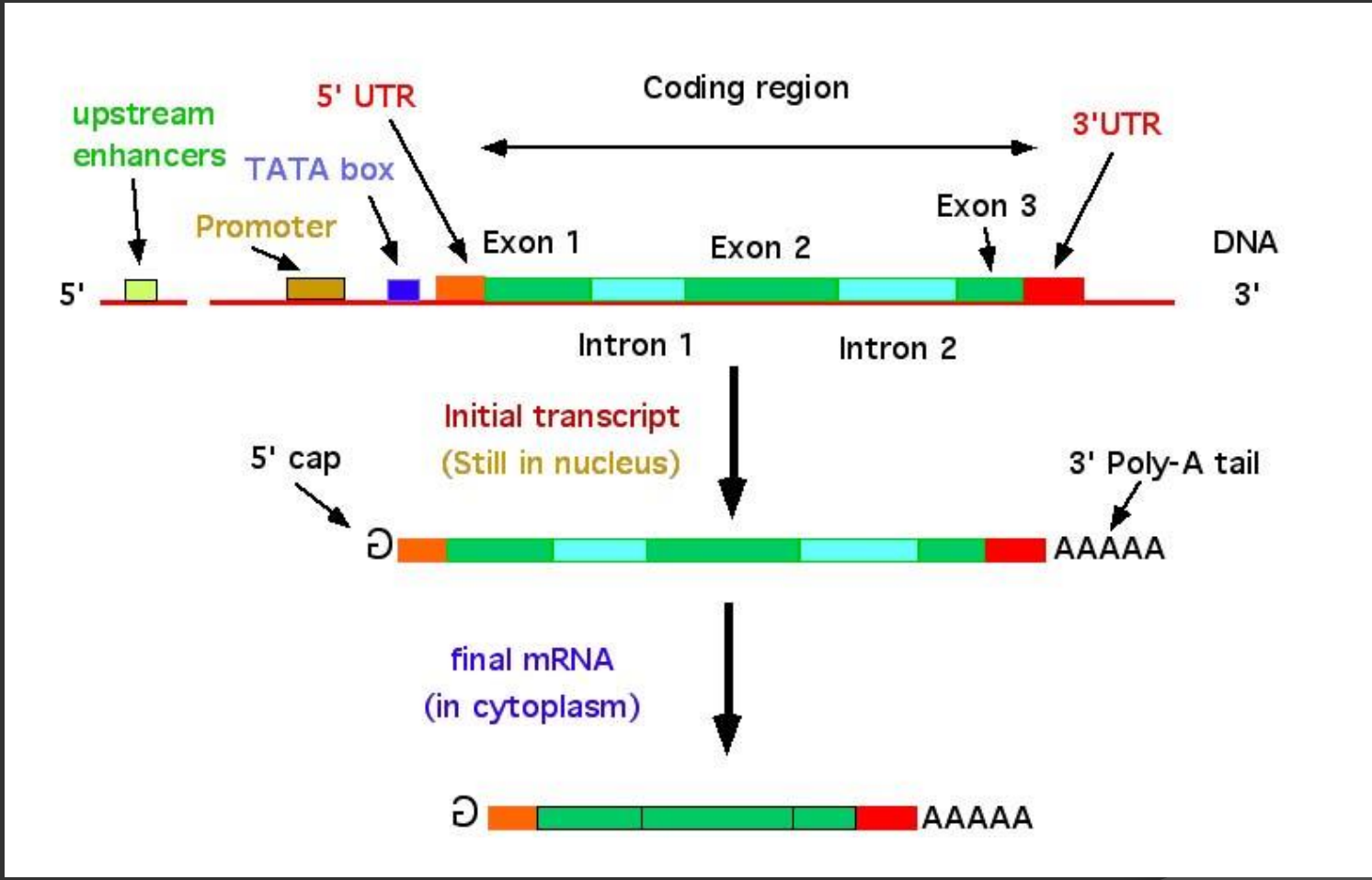
The basic problem

- Head and Neck neoplasm (HNN) are the 10th most common cancer in western society, the 6th most common cancer for both sexes and the 3rd most common cancer in third world countries (Parkin, et al 1980).
- Alcohol and smoking have been linked to HNN in over 75% of cases (Boyle et al 1995, Day et al 1993).
- Alcohol and tobacco act independently but **synergistically** in the development of HNN (Rothman et al 1972, Blot et al 1988).
- Human papilloma virus (svr 16, 18) is implicated in the non-alcohol/smoking HNN's.

Gene structure (very simplified)



4 bases are used in the genetic codon:
Adenine (A), Cytosine (C), Guanine (G), Thymine (T).

Gene Activation or Suppression

- Tumour suppressor genes.
  - They stop the growth of a cell.
  - Polymorphisms in these may increase cancer rates (breast cancer susceptibility genes BRAC-1, BRAC-2).
  - TP53 – tumour suppressor protein 53.
- Growth factor genes.
  - Initiate cell growth.
  - Epidermal growth factor

Reviewed in Walsh,J.E. et al Mechanism of tumour growth and Metastasis in Head and Neck Squamous Cell Carcinoma. CTOO 8:227-238, 2007

Types of Genetic Alterations in Cancer

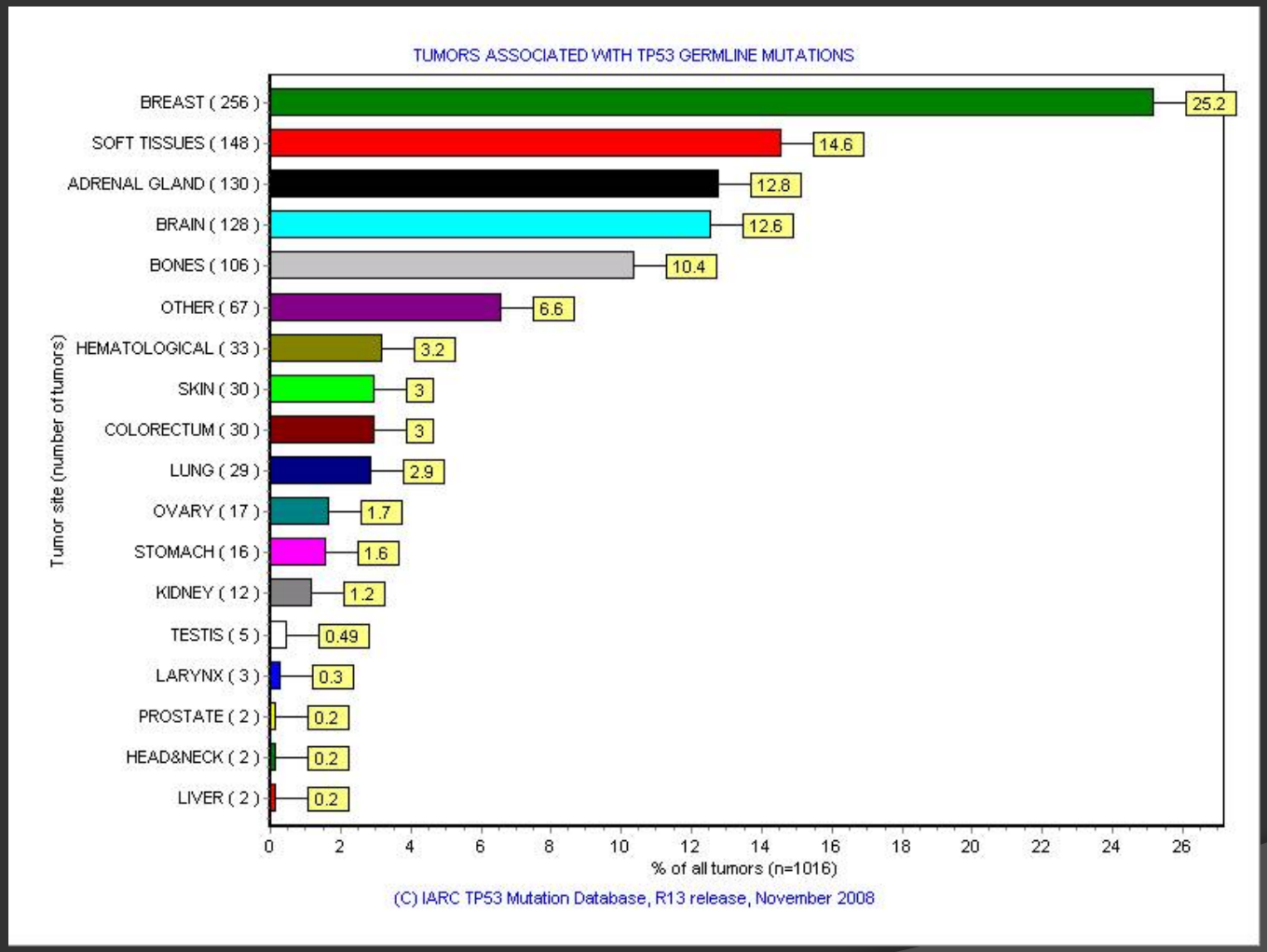
Somatic: Acquired during development and present only in cells undergoing clonal expansion

Inherited: present in the Germline and detectable in both healthy and cancer cells

- **Loss of parts or whole chromosomes**
- **Duplication of chromosomes**
- **Chromosome translocations**
- **Amplifications of chromosome fragments**

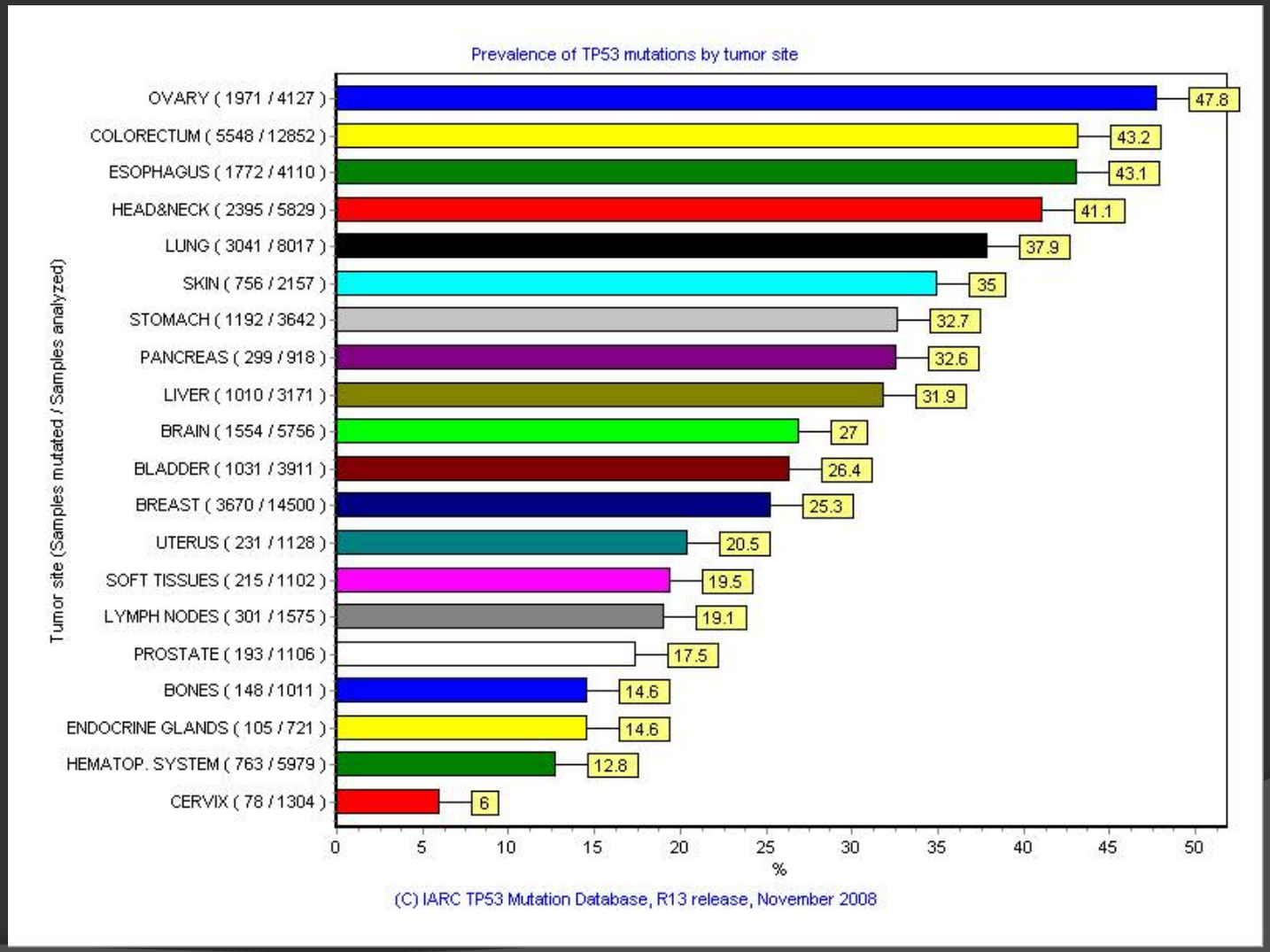
- **Iatrogenic deletions or insertions**
- **Recombination between adjacent genes**
- **Nonsense (Stop) mutations**
- **Missense mutations (substitutions)**
- **Methylation**

TP53 Germline Mutations Predispose To Several Types of Cancers



HNN p53 mutations are predominately somatic in origin (acquired)

TP53 Somatic Mutations are Frequent in Human Cancers



Carcinogenesis, Tumour Suppression/ Mutation.

- In oral SCCs several genes have been identified as the primary somatic mutation sites and they are seen in the early lesions.
- These sites need to include;
  - a mutation in or suppression of a tumour suppressor gene.
  - a mutation leading to or an increased growth factor expression.
- Once the carcinoma is initiated multiple and varied mutations are found within the cancer cells. Replicating virus rates are also increased once cell suppressor proteins are reduced – e.g. Epstein Barr Virus in oral SCC cells.

Reviewed in Walsh,J.E. et al Mechanism of tumour growth and Metastasis in Head and Neck Squamous Cell Carcinoma. CTOO 8:227-238, 2007

Tumour suppressor proteins associated with Oral SCCs.

Chromosome	Tumour Suppressor Genes
3p14.2 3p25-26	FHIT (Fragile Histidine Triad) VHL (von Hippel-Lindau tumour suppressor)
5q21	APC (adenomatous polyposis coli; high in Colonic CA evidence for Oral SCC poor)
8p21.3	LZTS1 (leucine zipper, putative tumour suppressor 1)
9p21.3	CDKN2A (p16) (Cyclin dependent Kinase 2a inhibitor/ p16) Influences TP53 expression.
13q14	**RB1** (Retinoblastoma susceptibility gene 1 - 50% of oral SCCs have down regulated activity.)
17p	**TP53** (40-60% of oral SCCs have down regulation)

Reviewed in Walsh,J.E. et al Mechanism of tumour growth and Metastasis in Head and Neck Squamous Cell Carcinoma. CTOO 8:227-238, 2007

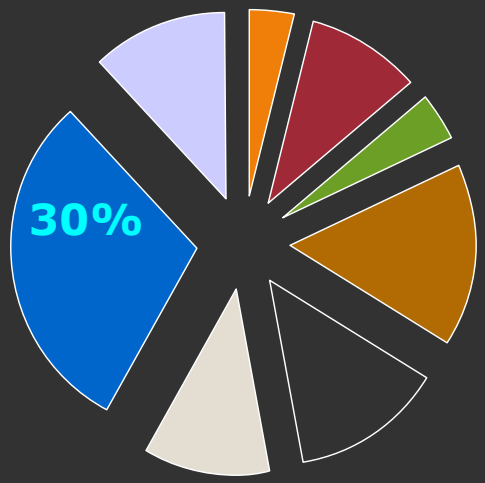
TP53 and Retinoblastoma proteins

- P53 is a DNA transcription inhibitor and stops the cell growth in certain parts of the growth cycle.
- Loss of P53 allows cell replication.
- RBP is an inhibitor of growth factor stimuli at both the nuclear membrane and within the nucleus.
- Loss of RBP allows increased cell growth

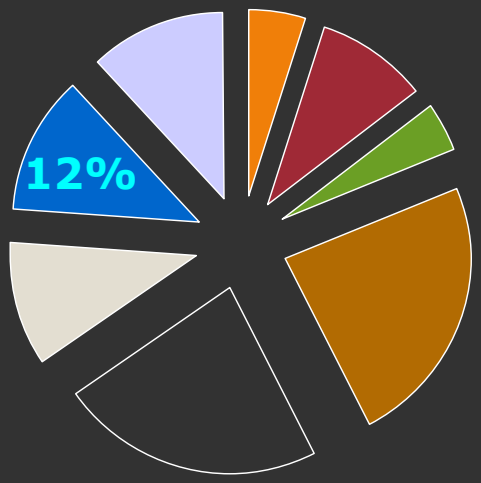
Reviewed in Walsh,J.E. et al Mechanism of tumour growth and Metastasis in Head and Neck Squamous Cell Carcinoma. CTOO 8:227-238, 2007

A Specific TP53 Mutation Pattern In Lung Cancer From Smokers

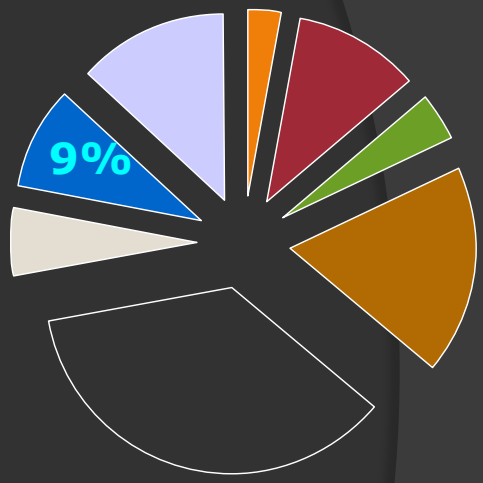
G:C>T:A



Smokers
(N=419)



Non-Smokers
(N=153)



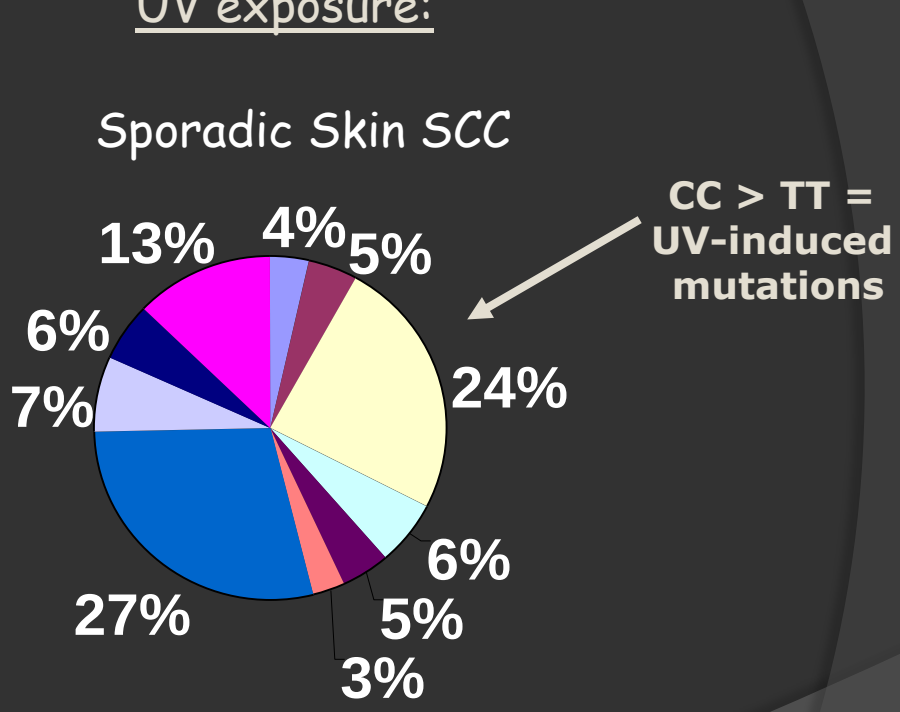
Non-tobacco related,
(N=4516)

G>T mutations are more frequent in lung cancers from smokers than in non-tobacco related cancers.

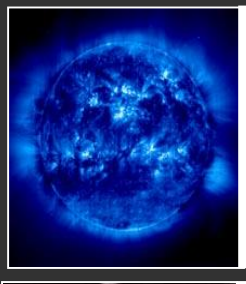
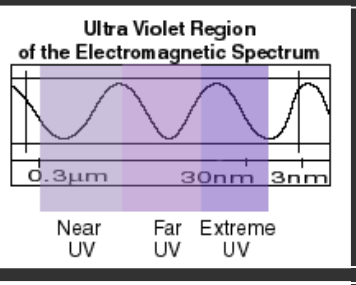
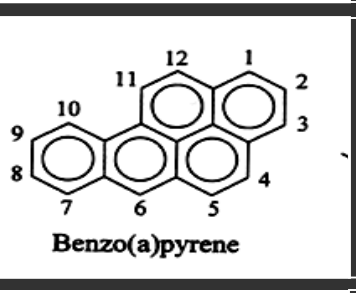
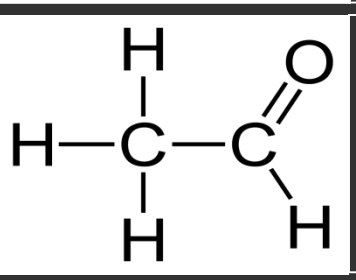
Pfeifer et al. Oncogene (2002)

TP53 Mutations In Skin Cancer: Effect Of UV Exposure

- Increase in CC mutations at sites where normally we see TT pairs.
- Cytosine bases are inserted in place of thymine bases.



TP53 Mutation Fingerprints in HNN

Source	Mutagen	TP53 mutation
UV radiation	 	CC to TT Various codons Skin cancer: 15% Other cancers: <1%
Tobacco smoke	 	G toT Codons 157, 158, 248, 273 Lung cancer: 30% Other cancers: <10%
Alcohol via Acetaldehyde	 	G to A Multiple Codons SCC oral, tongue Laryngeal cancer <60%

The pattern of mutations in the p53 protein are reasonably specific for the type of mutagen.

There is no distinct pattern of p53 mutations with HPV tumours.

Human Papilloma Virus & Oral SCC

- 65% of oral SCC's have HPV within the lesion and the presence of the virus is not related to alcohol or smoking frequency. (Giovannelli et al 2002)
- There has been increase in incidence of HPV related carcinomas since the 1980's.
- HPV16 and HPV18 are associated with oral SCC and also cervical carcinoma in females.
- No significant mutations found in P53 or Retinoblastoma proteins but P53 and BBP are rapidly degraded by the virus (Gimenez-Conti et al 1996; Huh et al 2008; Westra et al 2008).

HPV Risk factors

- No link with alcohol or smoking.
- Dietary link - reduced folate consumption.
- Life style : actinic radiation induced mutations may be linked.
- Genetic polymorphism links
  - Glutathione
  - Melanin (associated with actinic ray exposure)

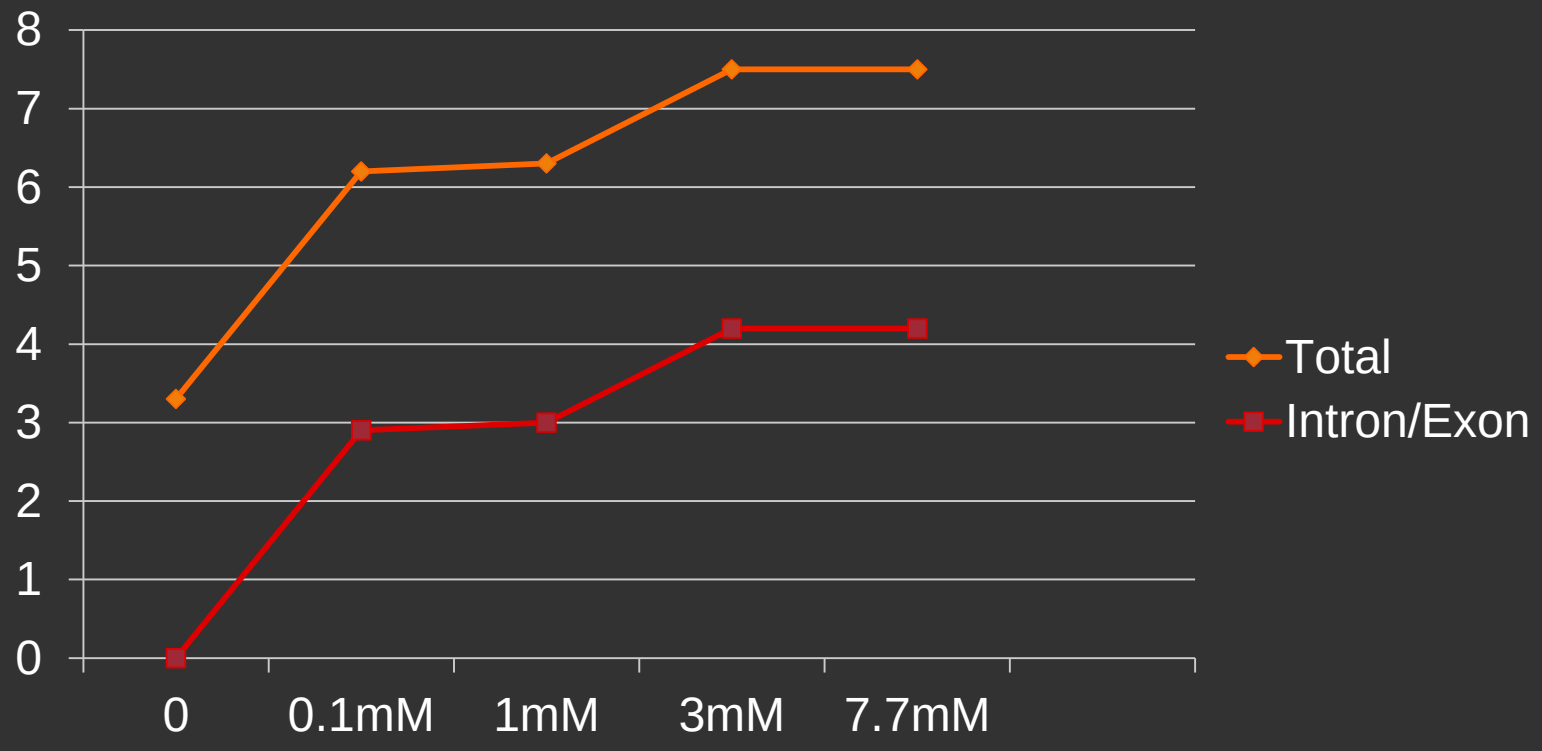
Mutations in Alcohol/Smoking - related Oral SCC's.

- Fundamental change is a mutation in tumour suppressor protein p53.
- Pattern of mutations are different for alcohol and smoking exposure related tumours.
- Alcohol induced mutations are associated with acetaldehyde induced mutations not ethanol.

Acetaldehyde & TP53 mutations

- Acetaldehyde induces mutations within the Intron/Exon sections. (Paget et al 2008).
- Acetaldehyde and its analogues bind to Guanine and interfere with polymerase Guanine base insertion into RNA (Paget et al 2008).
- Same pattern of mutations as seen in alcohol related SCC cells (Hemandez-Boussard et al 1998, IARC database).

Acetaldehyde mutations in TP53.



- Acetaldehyde induces the mutations at very low levels <1µM.
- Has a plateau effect above 0.1mM.
- Mutations occur at levels regularly seen in the mouth.

(Paget et al 2008)

Summary 1.

- Actinic radiation (lip), chronic smoke and acetaldehyde exposure are associated with SCC's in the face and upper aero-digestive tract.
- Dietary folate intake and gene polymorphisms in susceptibility genes are associated with increased incidence of SCC's.

Summary 2.

- HPV infection results in rapid intracellular degradation of p53 protein and has a similar effect as mutating the gene.
- Early lesions are associated with reductions in suppressor gene activity.
- Active lesion have increased growth factor expression.
- Malignant cells have mutations which result in uncontrolled cell growth.

The Opinions re Alcohol Mouthwashes.

- “There is now sufficient evidence to accept the proposition that developing oral cancer is increased or contributed to by the use of alcohol-containing mouthwashes.” (McCullough et al, 2008)
- “A range of recent critical and systematic reviews have failed to show any statistically significant association between mouthrinse use and oral cancer, despite factoring in genetic influences and other factors.” (Walsh, 2009)

Australian Mouthwashes

	Alcohol %
Listerine Antiseptic Mouthwash	27%
Listerine defence, Total, Smooth Mint, Fresh Burst, Cool Mint, Teeth Defence, Tartar Control	22.5%
Listerine Citrus burst	18%
Cepacaine	15%
Listerine Whitening (Includes H2O2)	8%
Listerine Mint & Zero, Reach, Savacol, David Craig, Diflam, Cepacol , Viodine, Oracol, Rainbow herbal mouthwashes	0%

Most companies have removed alcohol from their mouthwashes or introduced alcohol free mouthwashes since 2009. Data from Therapeutics Goods of Australia drug register 2011

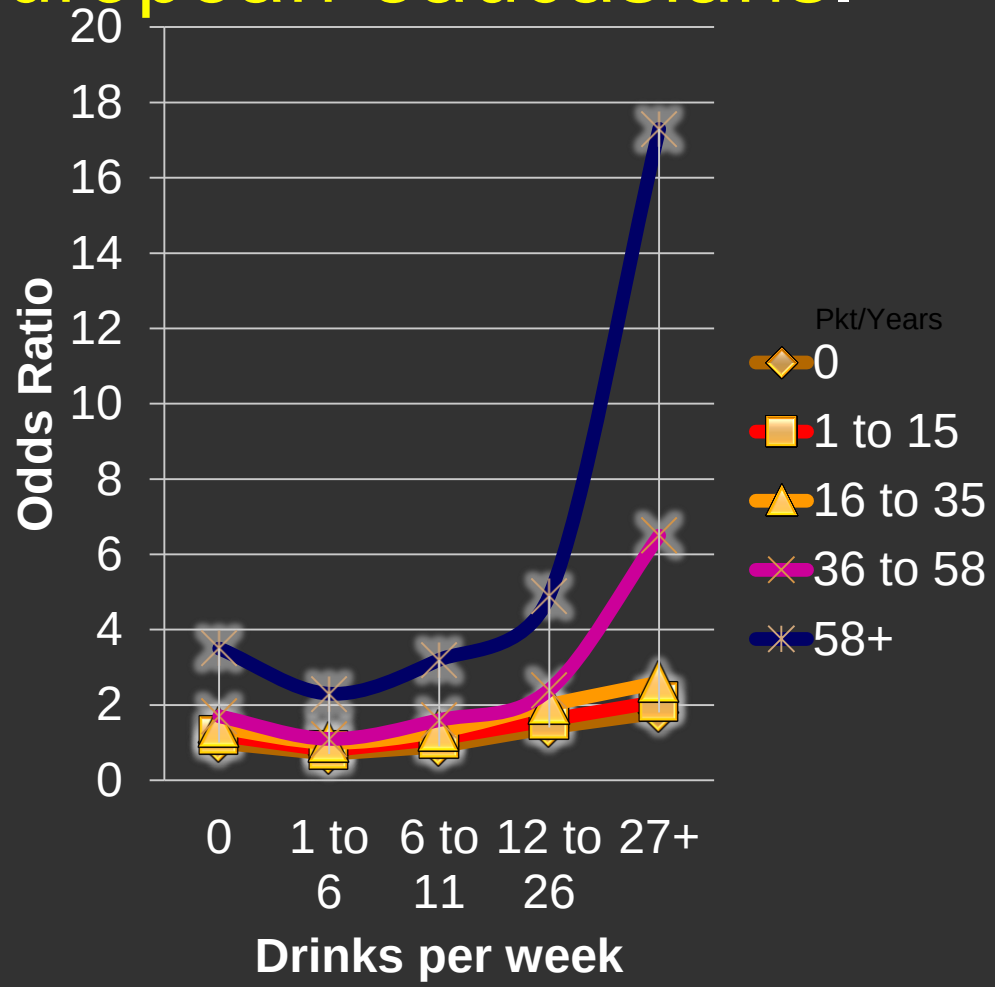
Promotion of Mouthwashes

- Recently published meta analyses (Gunsolley,J.C. 2010) conclude:
  - “There is strong evidence that supports anti-plaque, anti-gingivitis mouthrinses as effective agents. The benefits of the agents have sufficient value compared to current practices that they should be added to the oral hygiene regiments.”
- Many of the assessed studies have used the GI and PI indices (ordinal nonparametric data) and then analysed the data by parametric methods. One of the 7 deadly sins of medical statistics.
- The dental industry and panels of experts are promoting chronic mouthwash use.
- These studies are based upon a **one size fits all approach**.

Is Chronic use of Alcoholic mouthwashes associated with HNN?

- Eleven primary studies have been published.
- A mixed set of results have been achieved with some studies showing increased risk and others a reduced risk.
- Most poorly designed – all compared mouthwash use between test and control groups. One had an intra-group analysis.
- All studies showed an increased risk of HNN with poor oral hygiene and increasing plaque scores - **A microbial issue**?
- No study assessed the possibility of a synergistic effect with alcohol or tobacco exposure.
- Two studies showed an increased risk in US Blacks compared with US Whites with matching alcohol/smoking exposure (Day et al, 2003; Divaris et al 2010) suggesting genetic issues may be important.

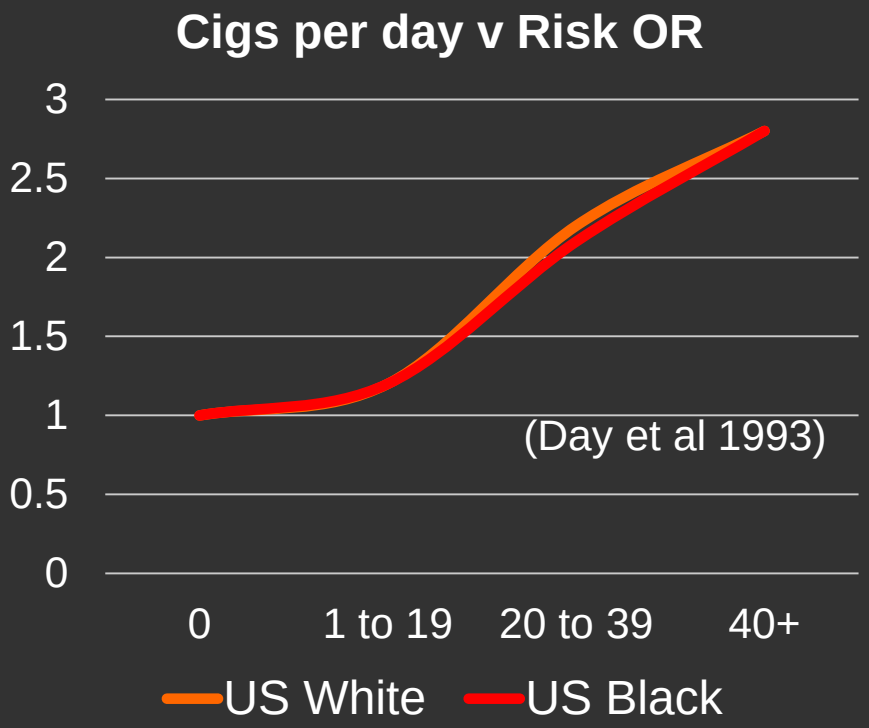
HNN risk with Alcohol and Smoking in European Caucasians.

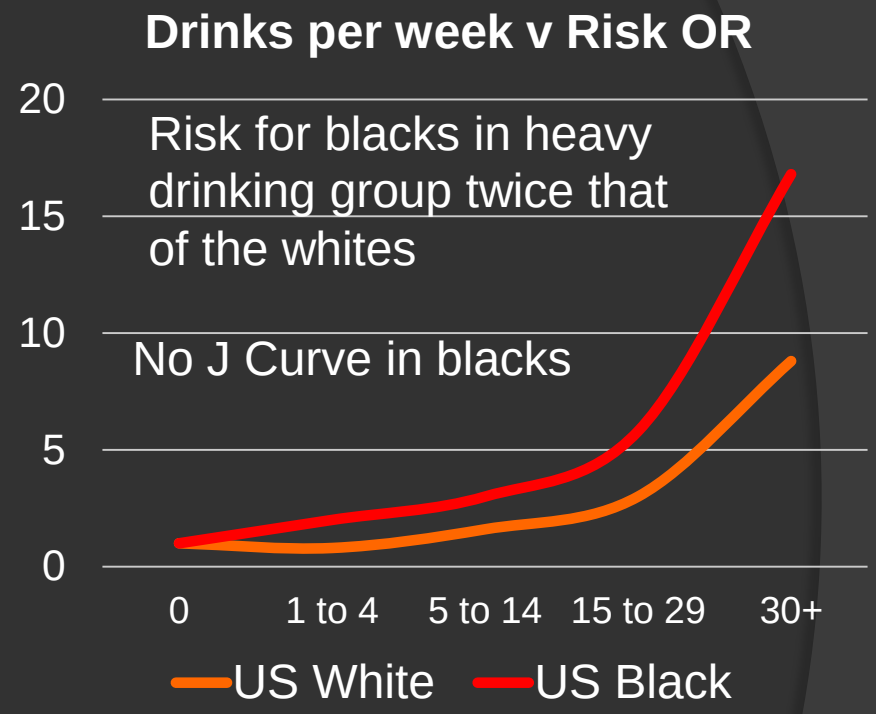


(Peters, et al 2005)

- Alcohol and smoking result in increased risk of development of HNN in a **"J" curve manner** in European Caucasians.
- 1-6 drinks/week **reduces the risk rate** for HNN in all categories of smokers.
- 6-11 drinks/week is no different from non drinking.
- **Is this "J" curve** response seen in subjects of Asian and African descent?

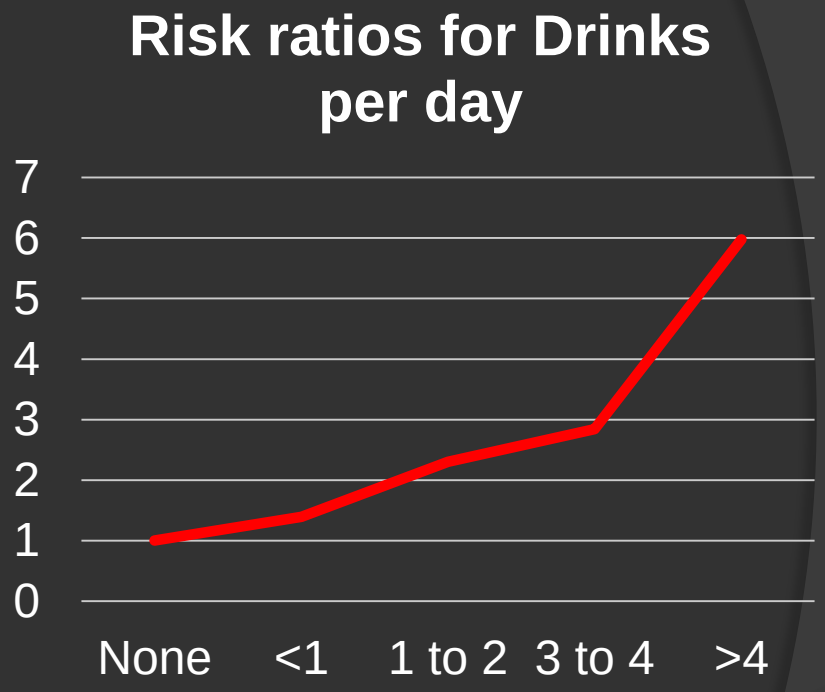
HNN Risk in American Blacks.





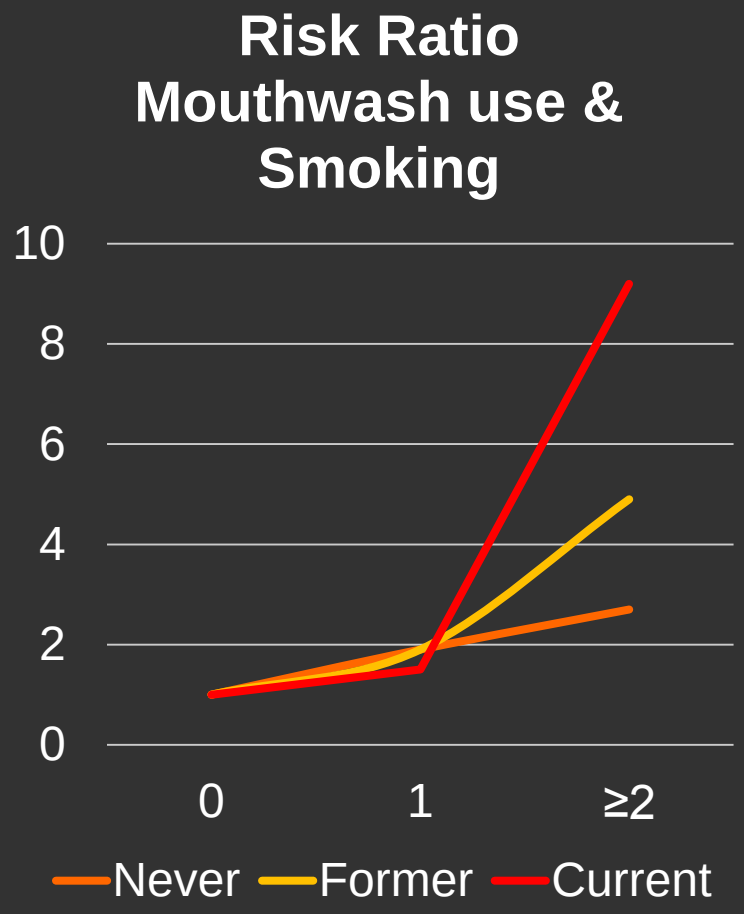
- Mouthwash risk: White = 1.3 (95%CL 1-1.6) ; Black = 1.6 (.9-2.8)
- Blacks have a 5 fold increase in oral SCC compared with Whites and the difference was not alcohol or smoking consumption related (Blot et al 1991; Brown et al 1997)
- Difference related to host genetics, microbiology or environmental factors.

HNN, Alcohol/Smoking in Chinese



No "J" curve response is noted in the Chinese (Fan et al 2008)
Thus considerable differences are noted between the races, with European Caucasians showing a reduction in risk with light Drinking which is not seen in the other races.

Mouthwash use and Smoking



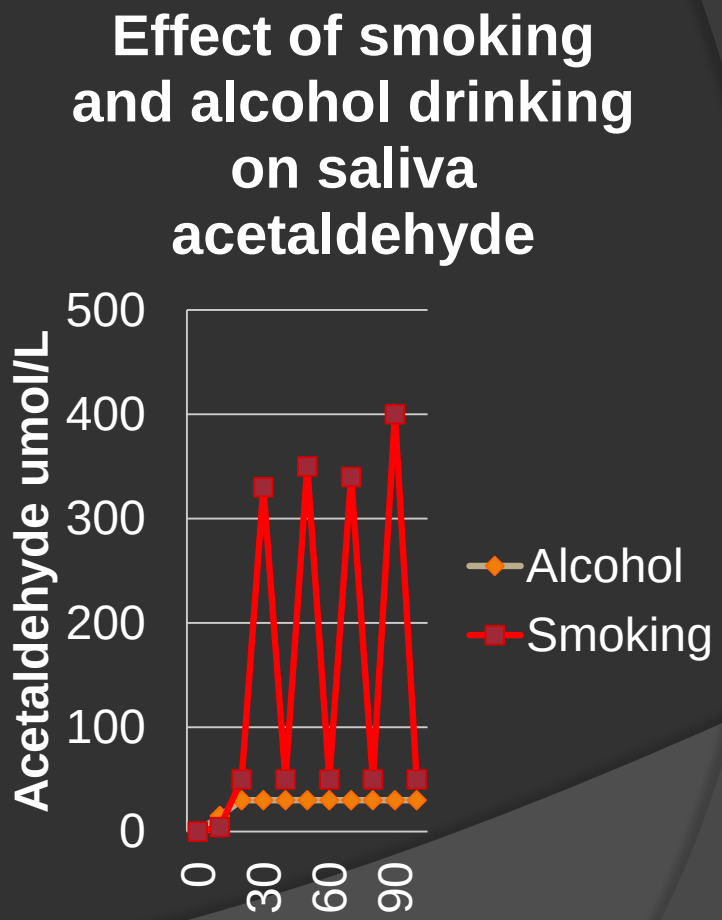
- The higher the usage of alcohol containing mouthwashes the higher the risk ratio for SCC.
- Risk ratios higher for current smokers than former smokers.
- A synergistic/ cumulative effect.
- (Guha et al 2007)

Tobacco, Alcohol & SCC

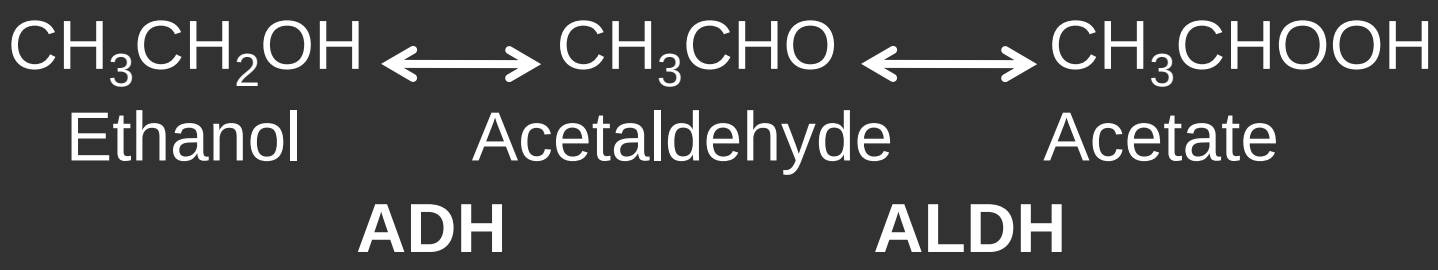
- Tobacco is a source of carcinogens and also provides acetaldehyde during the burning of the leaf (Smith et al 2000).
- Alcohol is metabolised to acetaldehyde as the second step in its degradation.
- Increased levels of acetaldehyde have been linked to DNA damage and mutations (carcinogenesis) through inhibition of Polymerase Guanine base insertion (Paget et al 2008).
- Acetaldehyde is higher in cancer patients and appears important in preventing cancer cell differentiation.
- Genetic alterations can influence acetaldehyde levels by increasing production or by reducing its removal.

Smoking and Acetaldehyde

- Burning tobacco leaves results in the production of acetaldehyde (Smith et al 2000).
- Salivary level of acetaldehyde as high as 400 µM/L can be measured after smoking a single cigarette (Salaspuro et al 2004).
- Synergistic effect on salivary acetaldehyde for smoking and alcohol.



Alcohol Metabolism



- ADH = Alcohol dehydrogenase (EC.1.1.1.1)
- ALDH = Aldehyde dehydrogenase (EC.1.2.1.3)
- This reaction also degrades xenobiotic aldehydes many of which may be carcinogenic and also removes acetaldehyde derived from dietary and microbial sources and smoking.

Alcohol dehydrogenase (EC.1.1.1.1).

- ADH is a dimeric zinc containing NAD-dependent enzyme, which has 5 subunits (α-ε) encoded by 7 genes (ADH1 to ADH7).
- The major alcohol degrading enzymes are from the class 1 ADH1 group.
- The ADH1 group has three enzymes (ADH1A, ADH1B and ADH1C).
- ADH1B has 3 polymorphic alleles *1, *2 & *3.
- ADH1B*2 is ~44x faster than ADH1B*1.
- ADH1B*3 is ~33x faster than ADH1B*1.
- ADH1C has 2 polymorphic alleles *1 and *2.
- ADH1C*1 is ~2.5x faster than ADH1C*2.

ADH1 polymorphism in Europeans

- Distribution
  - ADH1B*1 (slowest) (90%), ADH1B*2 (10%), ADH1B*3 (<1%)
  - ADH1C*1 (2.5x faster) (50%), ADH1C*2 (lowest) (50%)
- Acetaldehyde production/unit of time for polymorphic combinations given the same ethanol concentrations
  - ADH1B*1 + ADH1C*2 lowest (~45% of pop.)
  - ADH1B*1 + ADH1C*1 higher (~45% of pop.)
  - ADH1B*2 + ADH1C*2 higher (~5% of pop.)
  - ADH1B*2 + ADH1C*1 highest (~5% of pop.)
- The faster the conversion of alcohol to acetaldehyde the higher the risk of HNN and development of acetaldehyde related pathology, including liver disease and pancreatitis.

ADH1 polymorphism in East Asians

- Distribution
  - ADH1B*1 (30%), ADH1B*2 (44x fastest) (70%), ADH1B*3 (<1%)
  - ADH1C*1 (2.5x faster) (90%), ADH1C*2 (10%)
- Distribution of polymorphic combinations
  - ADH1B*1 + ADH1C*2 lowest (~3% of pop.)
  - ADH1B*1 + ADH1C*1 higher (~25% of pop.)
  - ADH1B*2 + ADH1C*2 higher (~7% of pop.)
  - ADH1B*2 + ADH1C*1 highest (~65% of pop.)
- The fast ADH1B*2 + ADH1C*1 combination is 5% of European Caucasians and 65% of East Asians.
- A greater genetic predisposition to produce acetaldehyde from any unit of ethanol occurs in East Asian subjects compared with European Caucasians

Aldehyde dehydrogenase (EC.1.2.1.3)

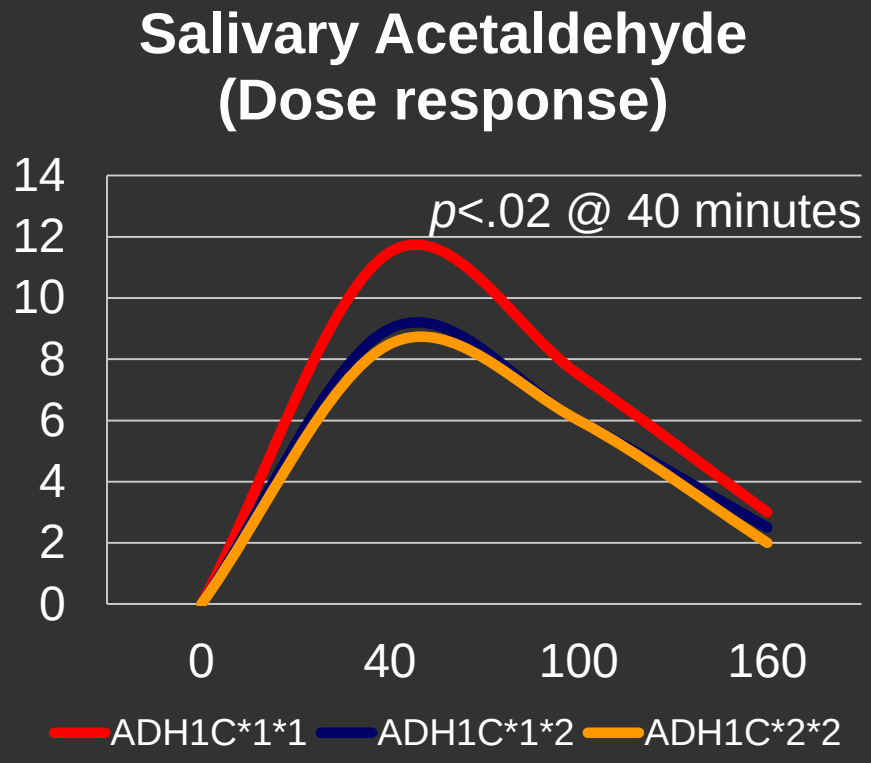
- 17 families of human aldehyde dehydrogenase (ALDH) enzymes exist (Reviews: Agarwal, 2001, Sladek, 2003).
- 3 of the 17 ALDH's are involved in alcohol metabolism but family 2 is predominate as it degrades ~90% of the acetaldehyde (reviewed by Dietrich 2006).
- ALDH1 are cytoplasmic enzymes.
- ALDH2 are mitochondrial enzymes.
- ALDH3 are inducible and respond to xenobiotic challenge.

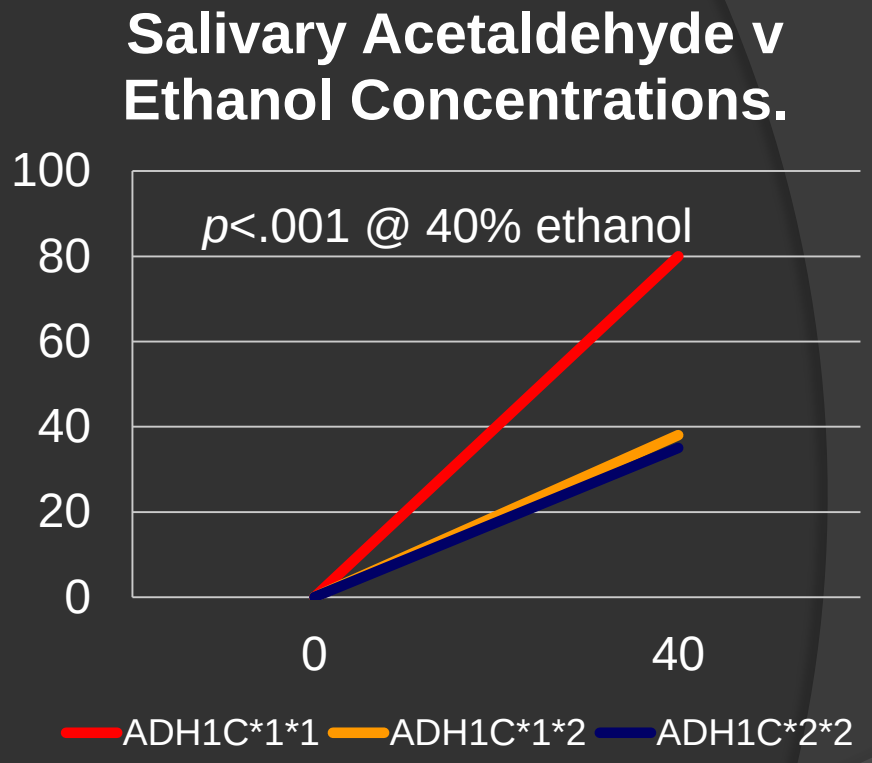
ALDH1 and ALDH2 polymorphisms

- Cytosolic ALDH1 has 3 polymorphic forms in Europeans (Linneberg et al, 2009).
  - One form (tt rs2073478) is slower and is homozygote in 1-2% of the population and heterozygote in ~20%.
  - The slow form is associated with acetaldehyde triggered histamine release (Alcohol sensitivity or Hangover) in the homozygote state ($p<.001$)
- Mitochondrial ALDH2 has 2 polymorphic forms in East Asians, *1 and *2.
  - 46% of East Asians (Cambodians, Vietnamese, Chinese, Korean, Japanese) carry the non-functioning ALDH2*2. 12-13% are homozygote.
  - The ALDH2*2 subjects usually avoid alcohol drinking due to the severity of their hangovers and facial flushing reactions.

Salivary Acetaldehyde & ADH1C

(Visappa et al 2006)





Significantly higher salivary acetaldehyde levels are noted in subjects homozygote for the 2.5x faster ADH1C*1. No differences noted for subjects carrying the slow ADH1C*2 allele.

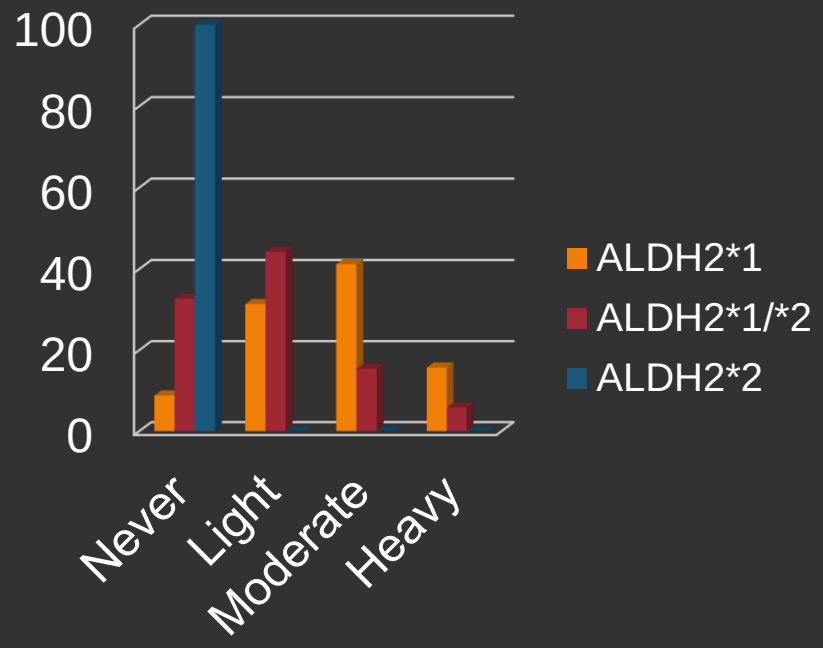
Blood Acetaldehyde & ADH1B/ALDH2

	ALDH2*1	ALDH2*1/*2	ALDH2*2
ADH1B*1	3.3±1.5	22.9±11.1*	-
ADH1B*1/*2	4.8±1.2	23.3±8.65*	-
ADH1B*2	4.2±1.3	24.1±12.8*	79.3±26.3

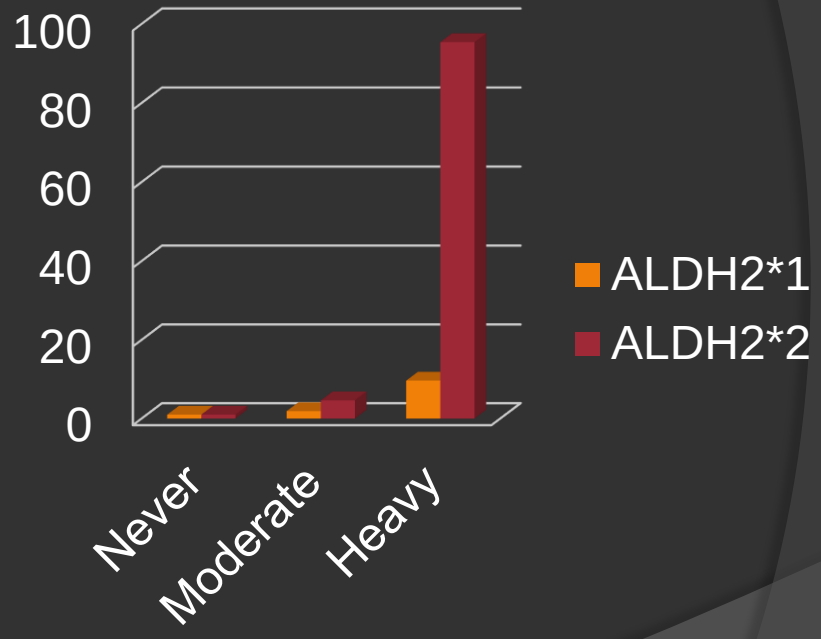
- 0.4 g/kg ethanol given to 68 healthy Japanese males after overnight fasting.
- ADH1B and ALDH2 alleles assessed.
- No differences noted with alteration of ADH1B alleles individually but $p<.04$ when ADH1B*1*1 homozygotes compared with ADH1B*2 carriers.
- Significant increases noted with all ALDH2*2 alleles ($p<.001$) The heterozygote subjects were intermediary.
- 24 fold increase in blood acetaldehyde (Mizoi et al 1994).
- The genetics of the host effects the acetaldehyde production rate.

ALDH2, Drinking & HNN risk

Drinking Behaviour and ALDH2 Polymorphisms



Risk Ratio for HNN and ALDH2 Polymorphisms



Whilst ALDH2*2 subjects rarely drink due to increased hangover symptoms, in those that do, the risk for HNN skyrockets (RRs as high as 350 in some studies). (Yokoyamma et al 2005, Yang et al 2005)

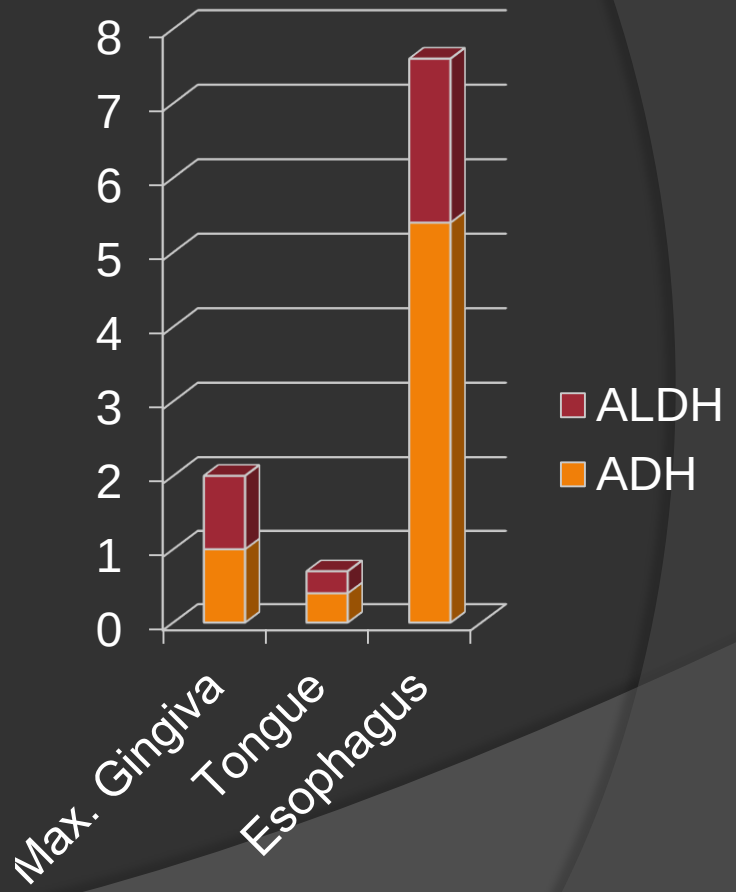
Distribution of Mouth SCCs (Tissue specificity).

	Male		Female	
Tongue	169	28.03%	122	35.57%
Lower Alveolar ridge	100	16.58%	65	18.95%
Buccal mucosa	92	15.26%	49	14.29%
Floor of mouth	76	12.60%	18	5.25%
Retromolar area	61	10.12%	28	8.16%
Maxillary Gingiva	53	8.79%	36	10.50%
Hard palate	40	6.63%	23	6.71%
Soft palate	12	1.99%	2	0.58%

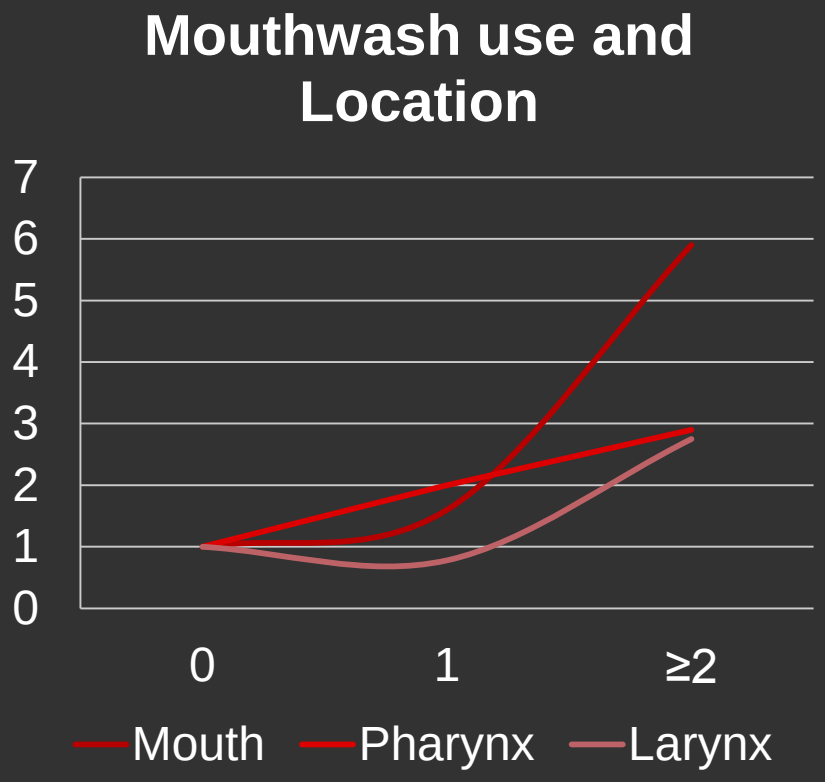
Spanish study

ADH/ALDH tissue distribution.

- Maxillary Gingiva: equal up-regulation of ADH & ALDH. **ADH:ALDH = 1**
- Tongue: ADH down to 40**%** of Gingiva. ALDH down to 30% of Gingiva. **ADH:ALDH=1.3**
- Oesophagus: ADH up 5.4 fold over Gingiva whilst ALDH up 2.2 fold over Gingiva. **AHD:ALDH=2.5.**
- Tongue has lowest rate of host acetaldehyde production and removal.
- Oesophagus has highest tissue production of acetaldehyde but a proportionally lower removal rate.
- The tissues with the highest levels of acetaldehyde have the highest incidence of SCCs.
- (Yin et al, 1993; Dong et al 1996)



Mouthwash use and SCC location.

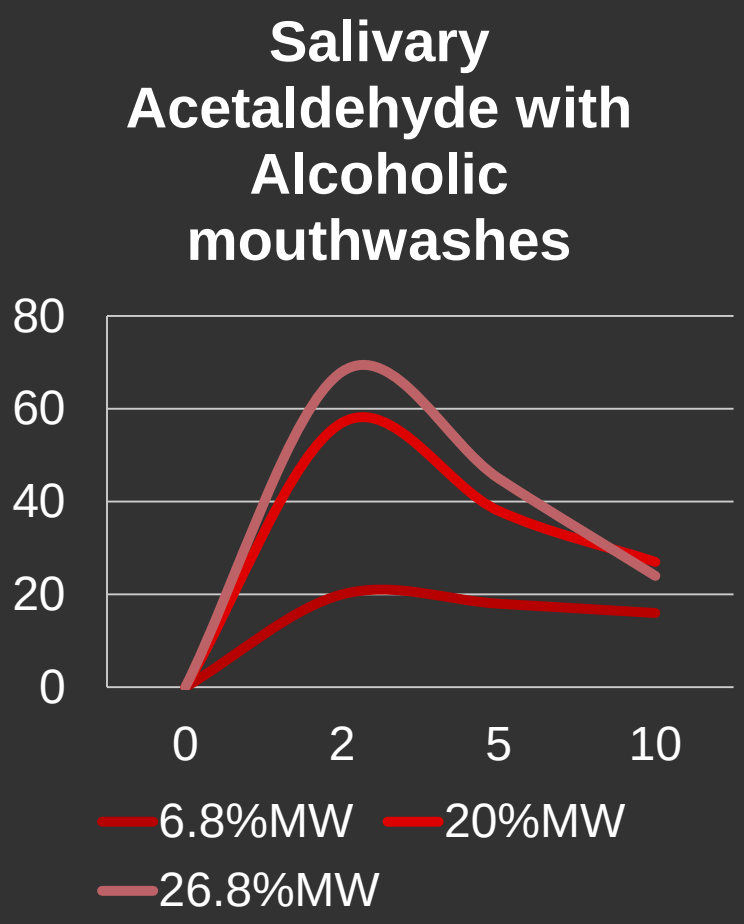


- An increase in mouthwash usage was associated with an increased SCC risk ratio and this varied with the location.
- The highest RR for mouthwash use was the oral cavity and the lowest the larynx (Guha et al, 2007).
- Those areas with the highest acetaldehyde exposure correlate with the highest RR

Microflora & Acetaldehyde (Homann et al 1997).

- The oral microflora make acetaldehyde from alcohol in a dose dependant manner. ($r=.94$ $p<.001$).
- Sterile or filtered saliva did not convert ethanol to acetaldehyde (hence Bacterial origin).
- Chlorhexidine (2x daily for 60 seconds) for 3 days significantly ($p<.01$) reduced saliva production of acetaldehyde but did not alter ethanol levels (the sample was taken 15 minutes after the mouthwash)
- The mouthwash used had 7% ethanol.
- Bacterial colony counts fell (Aerobes $2.6x10^8 \pm 1.4x10^8$ to $2.4x10^7 \pm 1.4x10^7$, $p<.01$).
- **The total salivary bacterial colony count did <u>NOT</u> correlate with acetaldehyde levels.**

Microflora & Acetaldehyde 2 (Yokoyama et al 1997).



- The higher the mouthwash alcohol content the higher the salivary acetaldehyde.
- The levels of acetaldehyde peaked at 2 minutes but persisted for greater than 20 minutes
- A 26% ethanol mouthwash produced a 3 fold increase in acetaldehyde over the 7% mouthwash.

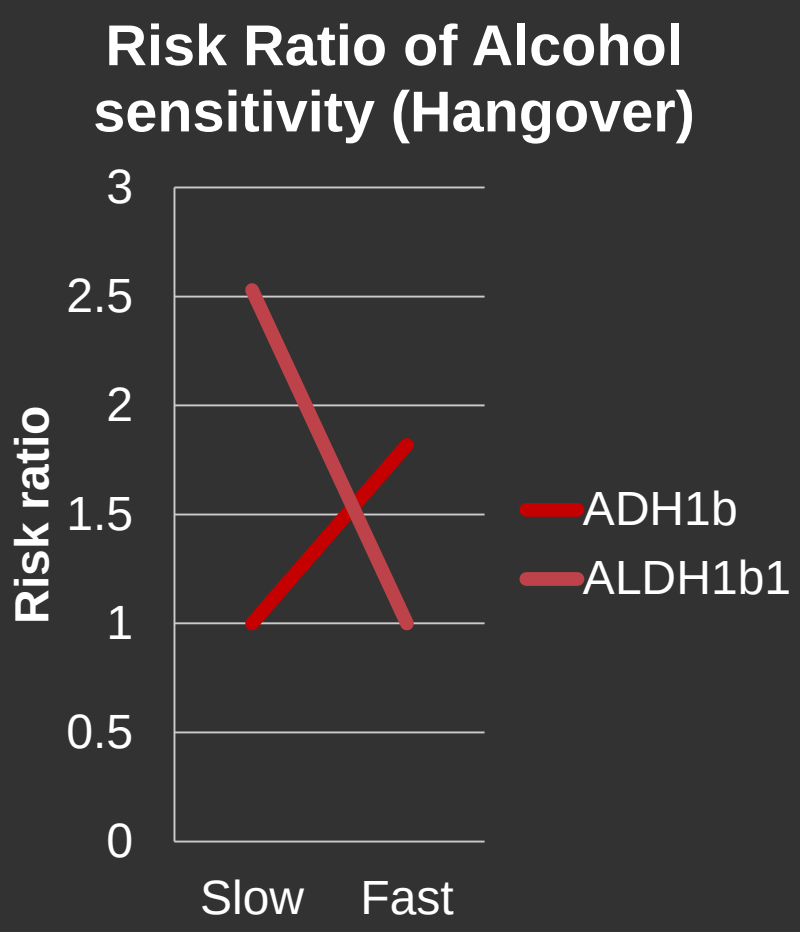
Microflora & Acetaldehyde 3.

- *Streptococcus anginosus, S.mitus, S.salivarius & Candida spp.* all produce acetaldehyde from alcohol.(Narikiyo et al 2004)
- α-haemolytic streptococcus counts are higher in alcoholics, smokers and in oral tumour patients (Narikiyo et al 2005)
- α-haemolytic streptococcus become the predominate oral microflora with chronic use of chlorhexidine mouthwash (Davies et al 1973; Borthen et al 1988). No studies performed with essential oils.
- α-haemolytic streptococci are found within most oral SCC tissues (Reviewed in Hooper et al 2009).

Bacterial distribution.

- Highest acetaldehyde producing species found in mandibular molar areas (Haffagee et al 2009).
- Lowest acetaldehyde producing species found in maxillary premolar/ Canine/ Incisor areas (Haffagee et al 2009).
- Distribution of highest acetaldehyde producing microorganisms coincides with the highest frequency of oral SCC occurrence.

Markers of higher Acetaldehyde: Scandinavians



- The carriage of the fast forms of ADH1b result in increased hangover symptoms.
- The carriage of the slow forms of ALDH1b1 result in increased hangover symptoms (Linneberg et al, 2009).
- Increases in acetaldehyde are associated with increased hangovers.

Markers of higher Acetaldehyde: East Asians

- Facial flushing with alcohol drinking
  - 46% of Asians (Japanese, Koreans, Chinese, Vietnamese, Cambodians) - ALDH2*2 gene
- Clinical Questions to assess flushing (ALDH2*2).
  - Do you develop facial flushing after drinking alcohol?
  - Did you develop facial flushing when you first started drinking alcohol?
  - **Yes to either strongly indicates ALDH2*2 with a high sensitivity/ specificity (~90%/~90%)** (Brooks et al 2009).
- **Recommendation**: Until studies can clarify the risk, subjects who exhibit signs of alcohol sensitivity should be advised to avoid **chronic** use of alcohol containing mouthwashes.

Symptoms:

- Rapid onset skin vasodilation (Facial flushing)
- Tachycardia
- Headache
- Nausea
- Hypotension
- Alcohol induced asthma
- Drowsiness

Conclusions 1

- Excessive Alcohol drinking and smoking are associated with increased risk of HNN.
- The effects of both are cumulative for increasing risk of HNN.
- Increasing levels of acetaldehyde via smoking, alcohol and genetic polymorphisms are associated with increased risk of HNN.
- Oral α-haemolytic streptococci and Candida spp are high producers of acetaldehyde when exposed to alcohol.

Conclusions 2

- Alcohol mouthwash use (≤2 x per day) may reduce SCC development risk in **non-drinking** subjects of Europeans origin who **do not** exhibit alcohol sensitivity.
- Chronic use of any form of alcohol containing mouthwash by subjects, with genetic risk of higher acetaldehyde production, may increase the risk of developing an oral SCC.
- **Risk factors should be assessed as CUMULATIVE and NOT assessed as INDIVIDUAL factors**, hence alcohol containing mouthwash recommendations made to any patient should be based upon a knowledge of the patients alcohol and smoking usage.

Conclusions 3

- Subjects with a significant **family history** of acetaldehyde associated cancers should not chronically use alcohol containing mouthwashes.
- Intermittent short term use of alcohol containing mouthwash is very unlikely to be associated with increased risk of SCC in any group.
- Whitening mouthwashes with hydrogen peroxide and alcohol require further research before they should be recommended for chronic use.
- More research is required to clarify these issues.

Questions

Is there a hidden meaning in this Ad?