

NCI Monograph 24

Tobacco and Alcohol: Understanding Co-Use to Reduce Cancer Incidence and Mortality

Chapter 5
**Physiological, Cellular, Molecular,
and Genetic Basis of the Combined Effects
of Tobacco and Alcohol Use on Carcinogenesis**

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Chapter 5

Physiological, Cellular, Molecular, and Genetic Basis of the Combined Effects of Tobacco and Alcohol Use on Carcinogenesis

Introduction

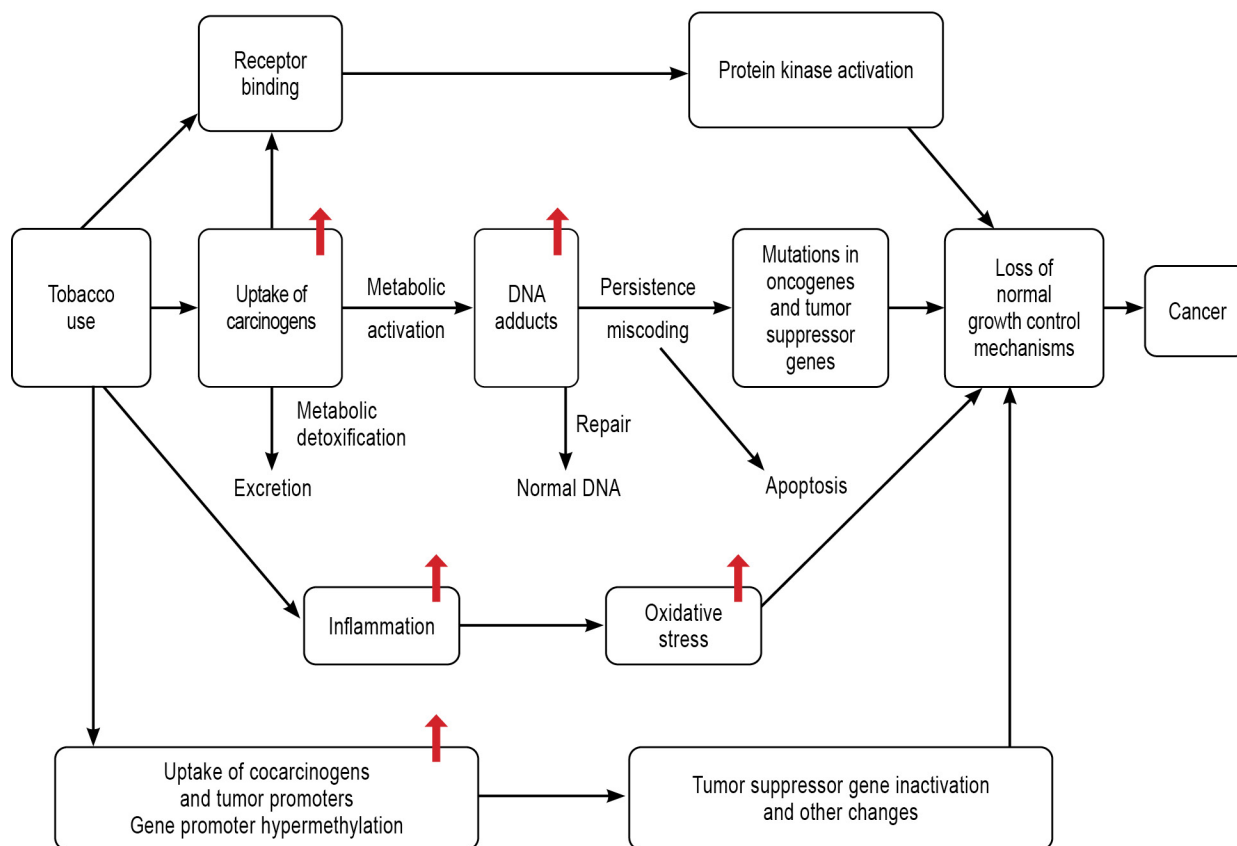
This chapter reviews research on the biological effects of tobacco and alcohol, used independently and together, and describes the underlying mechanisms that contribute to their carcinogenesis, including synergistic associations observed for some cancer sites. The chapter also highlights that much remains to be learned about these interactions, their combined roles in the initiation and/or promotion of cancer, and how these processes may be influenced by cessation of tobacco use and cessation or reduction in alcohol use.

As described in chapter 4, cigarette smoking causes cancers of the oral cavity, pharynx, larynx, esophagus, lung (including trachea and bronchus), stomach, liver, pancreas, kidney and ureter, bladder, cervix, colorectum, and blood (acute myeloid leukemia).¹ Suggestive evidence is also noted for other cancer types, including female breast,¹⁻³ although the totality of the evidence remains inconclusive for female breast cancer.^{1,3,4} As discussed in chapter 4, tobacco products other than cigarettes also cause cancer; for example, cigars cause cancer of the oral cavity, larynx, esophagus, and lung,⁵ and smokeless tobacco causes cancer of the oral cavity, esophagus, and pancreas.^{2,6} In the United States, cigarette smoking is responsible for an estimated 170,000 cancer deaths annually, accounting for 28.5% of all cancer-related deaths; in 2019, an estimated 19.3% of new cancer cases (~350,000) were attributed to cigarette smoking.^{7,8} The mechanisms underlying tobacco-related carcinogenesis have been studied extensively and have been summarized in numerous publications, including the U.S. Surgeon General's reports on smoking and tobacco use,^{1,9,10} National Cancer Institute reports and monographs related to use of cigars and smokeless tobacco,^{5,11} and several International Agency for Research on Cancer (IARC) monographs related to tobacco smoking and smokeless tobacco use.^{2,6,12-14}

The use of tobacco products results in exposure to numerous carcinogens both for the individual who uses tobacco and for other people via exposure to secondhand smoke. Carcinogen exposure is especially high from combusted tobacco products; smokeless tobacco use also involves exposure to carcinogens, with overlapping but distinct carcinogen exposures from these product types.^{2,6,15,16} Upon exposure, carcinogens from tobacco products react with and damage DNA, which can result in mutations occurring in critical genes leading to dysregulation of normal cellular growth and ultimately in cancer.^{2,9,17} DNA damage may also occur indirectly through the action of reactive oxygen species or the generation of lipid peroxidation products resulting from inflammation. However, mechanisms related to DNA damage are only one mode of action. Other nongenotoxic mechanisms related to the induction of inflammatory responses, epigenetic changes, and receptor-specific binding also make important contributions to the carcinogenic process. A general overview of these mechanisms is provided in Figure 5.1; a vertical red arrow

indicates mechanisms described for tobacco-related carcinogenesis that may be enhanced or affected by alcohol consumption.

Figure 5.1 Overview of Tobacco-Related Carcinogenic Mechanisms and Where the Influence of Alcohol Consumption Could Play a Role



Note. Mechanisms of tobacco-related carcinogenesis and processes where interaction with alcohol can occur (red arrows) result in an enhancement of the effects. Alcohol may increase the uptake of carcinogens or cocarcinogens by increasing the solubility of these compounds or by increasing the metabolism and activation of procarcinogens by inducing the expression of cytochrome P450 enzymes. Alcohol may contribute to increased inflammation, oxidative stress, and DNA damage directly through ethanol's major metabolite, acetaldehyde, or by increasing the activation of genotoxic compounds and DNA-damaging reactive electrophiles resulting from inflammation and oxidative stress. *Source:* Adapted from USDHHS 2014: Figure 6.4.¹

As will be discussed later in this chapter (“Potential Interactions of Electronic Cigarettes and Alcohol”), the potential carcinogenicity of electronic cigarettes (e-cigarettes) and other emerging tobacco products remains unclear. However, trends in e-cigarette use underscore the need for research in this area. Among adults in the United States, e-cigarette use increased from 4.5% in 2019¹⁸ to 7.0% in 2024.¹⁹ Although e-cigarette use among U.S. middle and high school students decreased from a high of 20.0% in 2019²⁰ to 5.9% in 2024,²¹ e-cigarettes remain the most commonly used tobacco product among adolescents in the United States.²¹

Alcohol consumption is also a known cause of cancer. In the United States in 2019, an estimated 5.4% of incident cancers (96,730 cases; 42,400 among men, 54,330 among women) were caused by alcohol consumption.⁷ Alcohol consumption was estimated to cause 4.1% of cancer deaths in the United States in 2019.⁷ Alcohol consumption has been classified as a Group 1 human carcinogen by IARC since 1988.²² This classification is based on a large body of epidemiological and mechanistic research. Increased risk in association with alcohol consumption has long been observed for cancers of the liver, oral cavity, pharynx, larynx, and esophagus. On the basis of studies reviewed in the 2010 IARC monograph, *Alcohol Consumption and Ethyl Carbamate*, alcohol is also thought to be a cause of female breast and colorectal cancers.²³ In addition, the World Cancer Research Fund/American Institute for Cancer Research has concluded that alcohol is a probable cause of stomach cancer,²⁴ although IARC has not updated its review of alcohol and stomach cancer. Possible associations between alcohol use and increased risk for cancer of the pancreas have also been observed.^{23,25} Major mechanisms for alcohol-induced cancer are both direct and indirect and include the following: (1) genotoxic effects of acetaldehyde, the primary metabolite of ethanol; (2) production of reactive oxygen or nitrogen species; (3) ethanol's ability to act as a solvent for multiple carcinogens, resulting in their greater absorption; and (4) influence of alcohol on folate and estrogen metabolism. Although much is known about the biological mechanisms through which alcohol induces cancer, these mechanisms are not yet fully understood and may vary depending on the target organ considered.

The fact that both tobacco and alcohol use increase the risk for several types of cancer, and that co-use of tobacco and alcohol is common, has motivated efforts to examine interactions between their use and cancer risk. The evidence for a synergistic effect of combined exposures to tobacco and alcohol is well established for cancers of the aerodigestive tract (oral cavity, pharynx, larynx, and esophagus).²³ Evidence from observational studies also suggests that co-use of tobacco and alcohol may result in synergistic effects on risk for colorectal, liver, lung, and female breast cancer.²⁶⁻³⁰ However, for these sites, it is not yet established if tobacco and alcohol interact to influence risk in those who use or have exposure to both substances. This evidence is discussed further in chapter 4.

Exposure to Tobacco- and Alcohol-Related Carcinogens

Co-use of tobacco and alcohol can result in biological interactions that influence initiation and/or promotion of carcinogenesis. Specifically, these interactions could result in sustained proliferation of abnormal cells, maintenance of tumor growth, and/or alterations in cell death processes.^{2,23} Tobacco-related carcinogenic mechanisms that may be influenced by alcohol consumption include increased exposure to carcinogens, particularly acetaldehyde; inflammation and oxidative stress; epigenetic changes; and tumor promotion (Table 5.1). Several metabolic and genetic factors may underlie these interactions and could result in different outcomes depending on the cancer sites considered, as well as the histological subtypes at different cancer sites. This chapter provides an overview of the carcinogenic mechanisms of tobacco and alcohol exposures derived from both mechanistic evidence and germline susceptibility. The review of evidence emphasizes aspects of interaction, where available, and discusses how alcohol may enhance or interfere with the main mechanisms described for tobacco carcinogenesis and vice versa.

Table 5.1 List of Tobacco-Related Carcinogenic Mechanisms Influenced by Alcohol and Relevance to Specific Cancer Sites

Candidate mechanism	Mechanisms of alcohol interaction	Relevant cancer site
Increased exposure to carcinogens	Increased exposure to acetaldehyde	Oral cavity and pharynx Esophagus Colorectum Lung ^a
	Increased carcinogen solubility/mucosal permeability	Oral cavity and pharynx Esophagus
	Effects on activation/detoxification of carcinogens	Liver Esophagus Lung ^a
Inflammation and oxidative stress	Additional production of reactive oxygen species and lipid peroxidation products and binding of receptors	Liver Esophagus Pancreas ^a Lung ^a
Epigenetic changes	Oncogene promotion and tumor suppressor silencing	Pancreas ^a Colorectum
Tumor promotion	Variation in expression of oncogenes or tumor suppressor genes	Esophagus Liver Lung ^a

^aThere is some evidence for an interaction between tobacco and alcohol use on carcinogenic mechanisms.

Source: IARC 2010,²³ IARC 2012,² Li & Jiao 2003,³¹ Li et al. 2019,³² Lachenmeier et al. 2017,³³ Malkinson 2005,³⁴ Seitz & Stickel 2007,³⁵ USDHHS 2010,⁹ Wight & Ogden 1998,³⁶ Yang et al. 2019.³⁷

The physiological studies of tobacco and alcohol co-use summarized in this chapter often focus on people who consume high levels of alcohol or on people with alcohol use disorder (AUD). Consequently, the physiology of tobacco use combined with moderate alcohol consumption is not as well understood. For example, fewer studies have investigated whether there are thresholds for identified mechanistic effects of tobacco and alcohol use. On the other hand, a growing body of large-scale genetic studies including a wide range of tobacco and alcohol use behaviors are starting to emerge (reviewed in chapter 6). Evidence concerning alcohol cessation or reductions in use and the effects of these behaviors on reversing carcinogenesis is modest for head and neck cancers and sparse for other cancers.³⁸ Therefore, this chapter attempts to generate hypotheses in this domain and highlight needed areas of investigation.

Finally, interactions between tobacco and alcohol at multiple levels, involving genetic, cellular, physiological, and behavioral processes, combine to affect use behaviors and cancer risk.³⁸ Chapters 4, 6, and 7 further discuss these interactions. This growing body of research supports addressing the co-use of tobacco and alcohol as a target for cancer prevention and control.

Exposure to Tobacco-Related Carcinogens

Tobacco smoke contains more than 7,000 chemicals, and at least 80 of them are recognized carcinogens.³⁹ Among these carcinogens, some classes of compounds are tobacco-specific, such as tobacco-specific nitrosamines, while others can also originate from other sources. Non-tobacco specific carcinogens include polycyclic aromatic hydrocarbons (PAHs); aromatic amines; volatile carcinogens such as formaldehyde, acetaldehyde, acrolein, 1,3-butadiene,

and benzene; and various metals. Most of these compounds are metabolized by enzymes that convert them to more water-soluble products that can be eliminated by the body. These conversions are mediated by enzymes such as cytochrome P450, glutathione transferase, UDP-glucuronosyltransferases and sulfotransferases, aldo-keto reductases, and N-acetyltransferases, and can also result in the production of reactive intermediates.⁴⁰ The consequent generation of electrophilic compounds or epoxides can result in species able to bind covalently with cellular targets and to damage DNA by forming DNA adducts. If not repaired, or if repaired incorrectly, these adducts can result in genetic changes affecting specific oncogenes or tumor suppressor genes and ultimately translate into abnormal cellular proliferation and carcinogenesis.

Exposure to Alcohol Carcinogens

Alcohol consumption refers to the drinking of wine, beer, and/or spirits, which have ethanol and water as main components in different proportions. These beverages are marked by different flavor profiles due to various congeners and volatile and nonvolatile compounds, deriving from the fermentation or distillation processes and from various added ingredients.²³ In addition, a wide variety of chemicals, impurities, and contaminants are found in alcoholic beverages, including known carcinogenic compounds such as PAHs⁴¹; N-nitrosamines, which have been detected in beer⁴²; and mycotoxins such as ochratoxin and aflatoxin, which have been found in some traditionally processed African alcoholic beverages.⁴³ By far the main actor in alcohol-related carcinogenesis is thought to be ethanol, as consistent differences in cancer risk among beer, liquor, and wine have not been found.^{24,44} There is wide agreement that ethanol is the major factor contributing to carcinogenesis related to alcohol consumption and that cancer risk depends on the amount of ethanol consumed regardless of the type of alcoholic beverage.^{23,24,44}

Alcohol and Increased Absorption of Tobacco-Related Carcinogens

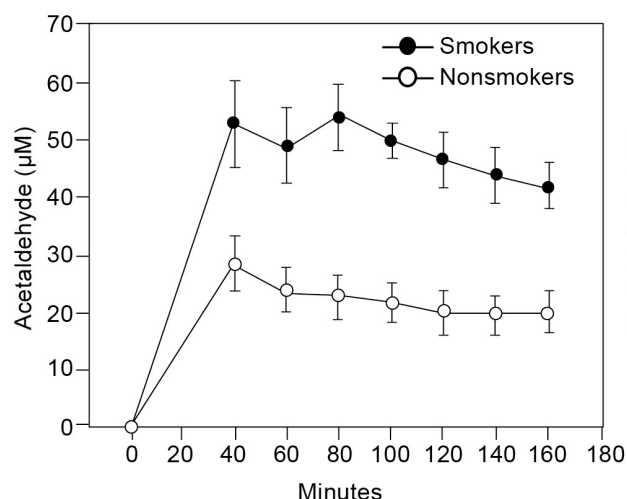
Alcohol consumption may contribute to an increase in the level of exposure to tobacco-related carcinogens by acting as a solvent and resulting in more efficient cellular absorption.⁴⁵ Additionally, alcohol may increase the permeability of the mucosa in the digestive tract, specifically the areas that come into direct contact with carcinogens resulting from exposure to both tobacco and alcohol compounds.^{36,46-48} A study on the effect of alcohol on pig oral mucosal permeability, using an *in vitro* perfusion model, showed an increase in the penetration of the tobacco-specific carcinogen N'-nitrosonornicotine (NNN) upon exposure to solutions of 25%–30% ethanol, but not of 50% ethanol, possibly due to higher concentrations of alcohol acting as a fixative and reducing membrane permeability.⁴⁹ For human ventral tongue, 15% ethanol increased mucosal permeability compared to 0% and 5% ethanol for tongue mucosa obtained at autopsy.⁴⁷ Additional studies are needed to determine alcohol's effects on mucosal permeability in the human oral cavity, and how this may influence tobacco-alcohol interactions and cancer risk.⁵⁰

More consistent evidence has been observed in studies that compared absorption of chromium-51-EDTA (51Cr-EDTA) in study participants with AUD and control participants. The 51Cr-EDTA method used examines the urinary excretion of orally administered chromium and allows a direct and accurate assessment of intestinal permeability in humans.⁵¹⁻⁵³

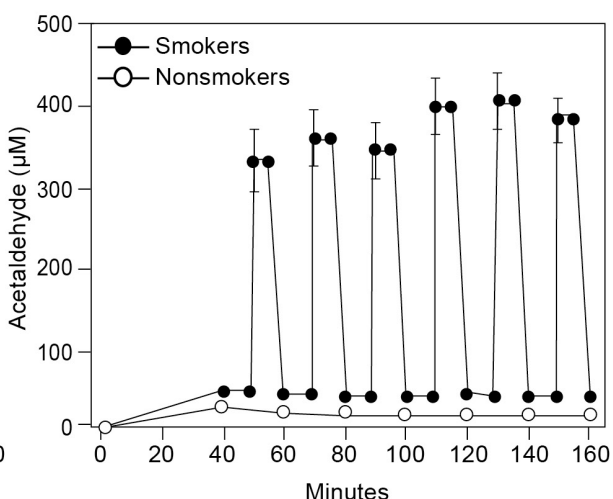
These studies show that alcohol disrupts the intestinal barrier, increasing its permeability by acting directly on the permeability of the epithelial cells and affecting the junctions between them. Alcohol and its metabolites induce oxidative stress, which damages the cells directly and weakens cell membranes while also disrupting the proteins that create the tight junctions linking cells and the proteins that stabilize the cells' cytoskeletons.⁵⁴ Such effects may be reversible. In one study, after 3 weeks of abstinence from alcohol, these effects disappeared, with levels of absorption of 51Cr-EDTA returning to those found in control participants.⁵³ In another study, among people with AUD and high levels of intestinal permeability, intestinal permeability decreased after 3 weeks of abstinence.⁵¹ A growing body of literature addresses effects of alcohol consumption, especially AUD, on gut microbial composition, inflammation, and the integrity of the intestinal barrier. This literature has implications for treatment of AUD and mechanistic understanding of the health effects of alcohol.^{46,48}

Role of Alcohol-Mediated Increase in Exposure to Tobacco-Related Carcinogens and Effects of Reduced Alcohol Intake

Several studies have examined the time course of changes in exposure related to the combined use of tobacco and alcohol. These studies are important because they provide a mechanistic underpinning for epidemiological observations of increased risk associated with the use and co-use of tobacco and alcohol. As discussed above and illustrated in Figure 5.2, salivary acetaldehyde increases dramatically with concurrent tobacco smoking and alcohol consumption. This relative increase could influence the risk for cancers of the upper gastrointestinal tract.⁵⁵ Salivary acetaldehyde levels are sensitive to the timing of tobacco and alcohol co-use and further characterization of the accumulation of acetaldehyde in saliva might help characterize the risk of co-use.⁵⁶

Figure 5.2 In Vivo Salivary Acetaldehyde After Ethanol Ingestion**Panel A: Acetaldehyde levels after ethanol ingestion**

A: *In vivo* salivary acetaldehyde levels after ethanol ingestion in people who smoke, without concomitant smoking, (black dots) and in people who did not smoke (white dots).

Panel B: Acetaldehyde levels after ethanol ingestion and active smoking

B: To measure the salivary acetaldehyde derived from the combined and simultaneous use of ethanol and tobacco, the same procedure was repeated in people actively smoking a cigarette. After ingestion of the ethanol dose, study participants smoked 1 cigarette every 20 minutes, and saliva samples were collected every 10 minutes for 160 minutes. The graph summarizes *in vivo* salivary acetaldehyde after ethanol ingestion in people who smoke, with concomitant smoking, (black dots) and in people who did not smoke (white dots).

Note. Study participants ($n = 6$ individuals who did not smoke; $n = 7$ individuals who smoked) were administered 0.8 g of ethanol/kg body weight, and salivary acetaldehyde levels were measured at several time points after ethanol ingestion. Differences between acetaldehyde concentrations are significant at all time points from 40 minutes to 160 minutes ($p \leq .05$).

Source: Reproduced with minor formatting changes from Salaspuuro and Salaspuuro 2004⁵⁵ with permission from *International Journal of Cancer* (publisher: John Wiley & Sons, Inc; Copyright © 2004 Wiley-Liss, Inc.).

In an experimental study of drinking cessation, the induction of cytochrome P450 2E1 (CYP2E1) by ethanol declined within 3, 8, and 15 days after alcohol consumption ceased.⁵⁷ Similarly, a mouse model has shown that 1 week after alcohol cessation, the effects of ethanol exposure on the hepatic imbalance of cellular redox homeostasis—regulated by the ratio of nicotinamide adenine dinucleotide, reduced nicotinamide adenine dinucleotide hydrogen (NADH) and nicotinamide adenine dinucleotide, oxidized (NAD⁺)—disappears.⁵⁸ Additionally, alcohol-induced gut inflammation can be moderated by the gut microbiome and dietary intake.⁵⁴ Multiple organs, including the gut, show recovery after cessation of alcohol use.⁵⁹ For example, after 3 weeks of abstinence, gut permeability largely recovers, inflammatory pathways are partially improved, and gut microbiota begin to return to more typical compositions.^{51,59} Together these mechanistic studies support the idea that cessation of alcohol consumption can reduce the risks associated with elevated exposure to acetaldehyde, to reactive species resulting from alcohol-induced enzymatic activity, and to alcohol-related increased gut permeability. A 2024 review of the epidemiological literature conducted by IARC concluded that there is

sufficient evidence that reduction or cessation of alcohol use reduced the risk of oral cancer and esophageal cancer (squamous cell carcinoma); limited evidence for reduced risk of larynx, colorectal, and female breast cancer; and inadequate evidence for reduced risk of pharynx and liver cancer.^{38,60} Studies including large numbers of people who stop drinking or reduce the amount they drink for multiple years are needed to determine the effects of cessation and reduction on cancer risk at sites where the evidence is currently limited or inadequate.

The Role of Acetaldehyde

Increased Levels of Exposure to Acetaldehyde

Multiple mechanisms are thought to underlie the causal relationship between alcohol and cancer; the strongest mechanistic evidence is for acetaldehyde, the primary metabolite of ethanol, which is also one of the main volatile compounds present in tobacco smoke.^{23,25,61} Consumption of both tobacco and alcohol products results in increased exposure to this genotoxic compound. Aldehydes in tobacco smoke are important carcinogens, inducing mutagenic PdG (1,N² propano-2'-deoxyguanosine) DNA adducts and impairing DNA repair, but also inhibiting some other procarcinogens in tobacco smoke from damaging DNA.⁶² Alcoholic beverages also contain acetaldehyde, and ethanol is metabolized into acetaldehyde in the body by alcohol dehydrogenase (ADH); acetaldehyde is further oxidized by aldehyde dehydrogenase (ALDH).⁶³ Hibberd and colleagues (2020)⁶³ review further details of the genetics and regulation of ADH in gastrointestinal and adipose tissues.

Acetaldehyde in alcoholic beverages is responsible for an initial increase in acetaldehyde in the saliva, but after a few minutes, conversion of ethanol into acetaldehyde dominates.⁶⁴ Acetaldehyde has been classified as a Group 1 carcinogen (indicating carcinogenic to humans) by IARC,²³ specifically in relation to head and neck and esophageal cancer through the induction of DNA damage. Additionally, the U.S. National Toxicology Program states that acetaldehyde is “reasonably anticipated to be a human carcinogen.”⁶¹

IARC’s Group 1 classification for acetaldehyde is further supported by the observation that individuals with genetic polymorphisms that reduce activity of aldehyde dehydrogenase 2 (ALDH2), for example rs671, which codes for an enzyme involved in detoxification of acetaldehyde, have an increased risk for alcohol-associated aerodigestive tract cancers.⁶⁵⁻⁶⁹ Individuals with one copy of the polymorphism have lower ALDH2 activity; if these individuals consume alcohol they have higher risks of cancer, especially of the aerodigestive tract, than individuals who lack the polymorphism. People with two copies of the polymorphism still have less detoxification of acetaldehyde. Although these individuals tend to not drink alcohol because the experience is extremely unpleasant, if they do drink alcohol, they have very high cancer risks. As described in chapter 4, 35%–45% of people of East Asian descent (an estimated 540 million people) have an ALDH2 polymorphism reducing acetaldehyde detoxification and increasing cancer risk, making this group an important target for alcohol-related cancer prevention efforts.⁶⁶

Role of the Oral Microbiome in Acetaldehyde Production

In the oral cavity, alcohol is metabolized mainly by the ADH enzymes present in cells of the oral mucosa and in oral microbes.⁷⁰ Metabolism of alcohol in the oral cavity is important for head and neck cancers, even though the liver is the primary site of alcohol metabolism.⁷¹⁻⁷⁴ The oral microbiome contributes substantially to the production of acetaldehyde in saliva, as shown by dramatically reduced acetaldehyde production after use of chlorhexidine (an antibacterial agent) mouthwash.⁷⁵ Although salivary acetaldehyde production and metabolism show high interindividual variation, there is a strong correlation between salivary ethanol and acetaldehyde levels after alcohol consumption.⁷⁵ Certain oral bacterial species, such as *Neisseria*, have very high ADH activity and acetaldehyde-producing capacity, and alcohol ingestion results in selection and increased production of these bacterial strains.⁷⁶ Bacteria of the oral mucosa present in saliva such as *Neisseria* species, *Rothia mucilaginosa*, *Streptococcus mitis*, *Streptococcus salivarius*, and *Prevotella histicola* can produce acetaldehyde from ethanol *in vitro*.⁷⁷ However, when a fixed amount of ethanol is added to whole samples of human saliva, acetaldehyde production is greater in the saliva with a lower abundance of *Neisseria* species.⁷⁸

These observations point to the complex role of the microbiome for ethanol metabolism in the oral cavity. This role is influenced by the considerable variability of the oral microbiota composition and by the balance of different species. Species representation and composition is influenced by exposures, including tobacco exposures.^{70,79-81} Salaspuro and Salaspuro (2004)⁵⁵ demonstrated that after ethanol ingestion, people who smoke have higher salivary acetaldehyde levels than people who do not smoke, and that salivary acetaldehyde production deriving from ethanol consumption is increased by active smoking. Specifically, as shown in Figure 5.2, after a dose of ethanol, the mean *in vivo* salivary acetaldehyde level in people who smoke was observed to be two times higher than in those who did not smoke. When the ethanol challenge was repeated during active smoking, the levels of salivary acetaldehyde were seven times higher in participants who smoked than in those who did not smoke. The authors suggested that these findings may be due to “microbial changes in the oral cavity of smokers, to inhibition of ALDH of the oral mucosa by tobacco smoke, and to the significant deposition of acetaldehyde from tobacco smoke in the saliva.”⁵⁵ This study suggests a potential mechanism for the interactive effects of tobacco and alcohol co-use: substantially increased acetaldehyde exposure that results from oral alcohol metabolism combined with the high levels of acetaldehyde present in inhaled tobacco smoke.⁵⁵

Acetaldehyde-Derived DNA Damage

Somatic genomic studies have also been informative regarding carcinogenic mechanisms mediated by acetaldehyde-derived DNA damage. Acetaldehyde, a reactive metabolite, can covalently bind to DNA, forming monoadducts, such as *N*²-ethylidene-dG or 1,*N*²-propano-deoxyguanosine, and DNA and protein crosslinks. These modifications trigger different repair pathways and differ in their mutagenic potential and effects on carcinogenesis.⁸² Recurrent mutation patterns, or DNA mutational signatures, have been linked to exposure to alcohol⁸³ and DNA damage in stem cells.⁸⁴ Genetic polymorphisms that inhibit the function of ALDH2 appear to modulate exposure to carcinogens (including acetaldehyde) and their impact on the somatic genome of carriers.^{85,86} For example, a study examined levels of acetaldehyde-derived DNA monoadducts by *ALDH2* genotype in peripheral blood samples collected from individuals who were admitted for treatment after diagnosis of AUD.⁸⁷ The level of

three acetaldehyde-derived DNA adducts was found to be substantially higher in blood samples from patients with AUD who were carriers of the *ALDH2* polymorphism than in samples from patients with AUD who were noncarriers, providing direct evidence that the *ALDH2* genotype affects the acetaldehyde-related genotoxic damage.

Additional insight is provided by studying patients with Fanconi anemia (FA), a rare genetic disorder characterized by impaired DNA repair. The FA repair pathway is the major mechanism counteracting the damage caused by endogenous and exogenous carcinogenic aldehydes (including acetaldehyde) by avoiding the resulting interstrand crosslinks from degenerating into double-stranded breaks.^{84,88,89} As a consequence, patients with FA are at higher risk for developing certain types of cancers. For example, the incidence of head and neck squamous cell carcinoma in patients with FA is 500 times higher than in the general population.⁹⁰ These observations further point to the carcinogenic role of acetaldehyde through its genotoxicity. They also support increased carcinogenicity due to a higher level of exposure with tobacco and alcohol co-use. Future studies of people with genetic variants can provide novel and important insights into causal mechanisms of carcinogenesis.

Further Mechanisms of Tobacco and Alcohol Carcinogenesis

Perturbation of Carcinogen Metabolism

Alcohol may interfere with the metabolic degradation of tobacco-related carcinogens. The most consistent evidence to support this hypothesis relates to the effects of ethanol on exposure to carcinogenic tobacco-specific nitrosamines that can be formed from tobacco products. Swann (1984)⁹¹ demonstrated that alcohol inhibits the hepatic metabolism and clearance of N-Nitrosodimethylamine (NDMA). This inhibition occurs because ethanol competitively inhibits hepatic CYP2E1, the main hepatic enzyme responsible for the metabolism of NDMA. Consequently, higher amounts of NDMA reach extrahepatic tissues where the nitrosamine can be metabolically activated to reactive species with the potential to interact with cellular targets and disrupt their function.^{91,92} Similarly, chronic alcohol consumption induces cytochrome P450 2A6 (CYP2A6), the enzyme primarily responsible for the metabolism of nicotine, leading to an increased rate of nicotine metabolism.⁹³ This effect in turn could lead to compensatory increases in tobacco use, thereby increasing exposure to carcinogens in tobacco smoke. One study found that CYP2A6 levels were elevated in liver biopsy specimens of individuals with histories of excessive alcohol consumption.⁹⁴ Other studies found that alcohol induced CYP2A6 in the U937 macrophage cell line exposed to alcohol⁹⁵ and in the mouse orthologue of human CYP2A6—CYP2A5—in mice exposed to alcohol.^{96,97} These findings are consistent with the observation that the increased rate of nicotine metabolism in men undergoing treatment of AUD declined 4–7 weeks following cessation of alcohol use.⁹⁸

Alcohol-Induced CYP2E1 and Activation of Carcinogens Present in Tobacco Smoke

Chronic alcohol consumption increases the rate of ethanol elimination by the induction of CYP2E1 activity.⁹⁹ This induction results in increased production of reactive oxygen species, augmenting those produced by tobacco use while also more efficiently converting procarcinogens present in tobacco smoke to active carcinogens. Ethanol intake has been shown to induce these carcinogen-activating enzyme systems in the liver, the esophagus, the lungs, and the intestine, which are all target organs for tobacco-related cancers.^{100,101}

Effects of Alcohol on Retinol Metabolism and Other Metabolic Dysfunctions

Alcohol consumption can reduce hepatic retinol (vitamin A) levels, either by increasing its mobilization from the liver to extrahepatic regions or by increasing its degradation through the induction of the cytochrome P-450 2E1 (CYP2E1) metabolic systems.¹⁰² Additionally, retinol and ethanol have similar metabolic pathways, and ethanol can act as a competitive inhibitor of vitamin A oxidation to bioactive retinoic acid involving ADHs and ALDHs.¹⁰³ Similarly, tobacco smoke interferes with retinoid metabolism and reduces its plasma retinol levels.¹⁰⁴ Retinol plays a fundamental role in maintaining normal cellular growth and differentiation mechanisms.¹⁰⁵ Furthermore, retinol has been shown to have anticarcinogenic properties that interfere with both the initiation and promotion of the carcinogenic process; therefore, reduced retinol levels can contribute to the disruption of retinol-mediated signaling, leading to uncontrolled cellular proliferation and carcinogenesis.¹⁰⁵

Finally, AUD can lead to nutritional deficiencies.¹⁰² These nutritional deficiencies include folate deficiency, a vitamin deficiency that can contribute to an inhibition of methyltransferases, enzymes that are important in gene regulation. Additionally, AUD may also result in zinc deficiency,¹⁰⁶ and low levels of this essential vitamin are associated with adverse health outcomes including impaired immune system function with a possible role in carcinogenesis.¹⁰⁷

Inflammation and Oxidative Stress

Inflammation and induction of oxidative stress are two distinct processes that may play independent roles in carcinogenic processes.¹⁰⁸ In the context of tobacco- and alcohol-related mechanisms of interactions, the focus of most research has been on the ability of both substances to induce inflammation resulting in lipid peroxidation and production of reactive oxygen species.

Cigarette smoke contains substances that can induce inflammation resulting in increased cellular proliferation, activation of nuclear factor-kappa B (NF- κ B), and tumor promotion.⁹ The promotion of oxidative stress by the generation of reactive oxygen species and the depletion of endogenous antioxidants may account for some of the increased risk of hepatocellular carcinoma associated with smoking.¹⁰⁹

Alcohol consumption has been shown to result in the induction of several hepatic metabolic changes resulting in oxidative stress.¹¹⁰ The induction of CYP2E1 is a source of production of reactive oxidative species. Furthermore, depletion of the antioxidant glutathione in the setting of chronic alcohol exposure in people with AUD can also increase cellular oxidative stress.^{111,112} Additionally, alcohol consumption has been associated with the upregulation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidases^{113,114} and mitochondrial oxidative stress.¹¹⁵ Consequently, simultaneous exposure to tobacco and alcohol is expected to concurrently promote the role of these processes in increasing the risk for developing liver cancer.¹⁰⁹

People who smoke experience proinflammatory changes in their lungs,^{34,116} and studies in laboratory animals demonstrate that anti-inflammatory agents can decrease tobacco carcinogen-induced lung tumorigenesis.¹¹⁷ In addition, chronic obstructive pulmonary disease (COPD), especially emphysema, is an independent risk factor for lung cancer in individuals who smoke.¹¹⁸ Because COPD causes inflammation in the airways, this association further implicates the role of inflammation in lung cancer.^{118,119} The mechanisms associated with these results are

based in part on the fact that inflammatory processes result in lipid peroxidation and generation of reactive oxygen species which can damage DNA and result in mutations. Additionally, products from these processes can result in the induction of cyclooxygenases (COXs) which can lead to the production of prostaglandins that are thought to be important mediators in solid tumor carcinogenesis.¹²⁰

Individuals with AUD have an increased risk of both the occurrence and severity of lung infections, including pneumonia, tuberculosis, respiratory syncytial virus, and acute respiratory distress syndrome.^{121,122} These infections could lead to exacerbations of chronic inflammatory lung diseases, which increase the risk of lung cancer in people who smoke. Epidemiological evidence for associations between alcohol and lung cancer remains mixed, with some studies suggesting an increased risk of lung cancer associated with drinking alcohol¹²³ and others reporting inverse associations of alcohol consumption.^{124,125} Further evidence concerning lung cancer and co-use of tobacco and alcohol is discussed in chapter 4.

The interaction of tobacco and alcohol in inducing inflammation and oxidative stress has also been suggested to play a role in increasing the risk of pancreatic cancer.¹²⁶ Oxidative DNA damage and lipid peroxidation-induced DNA adducts have been found in the pancreas in association with increased *KRAS* mutations in tissues from people with and without pancreatic cancer. These mutations have also been found to occur in both people who smoke and people who use alcohol.^{31,127} However, further analyses have mixed results with only some studies reporting associations between alcohol consumption and pancreatic cancer,^{23,128,129} mostly with higher levels of alcohol consumption.^{23,130-132} Interpretation of these results is also complicated by the potential for confounding by smoking or exposure to secondhand smoke and by dietary variables related to inflammation. Nevertheless, higher levels of alcohol consumption appear likely to be associated with pancreatic cancer.¹³²

Epigenetic Changes

Epigenetic changes, defined as heritable modifications to gene expression that are independent of changes to DNA sequences,¹³³ are associated with tobacco and nicotine exposure.¹³⁴ Such changes are most apparent in people who smoke cigarettes, and some may be reversible upon smoking cessation, raising the possibility of using patterns of methylation or other epigenetic changes as biomarkers to distinguish between current, former, and never smoking.¹³⁵ Additionally, the oxidative damage and gene promoter methylation processes induced by cigarette smoking are an integral part of cancer progression. Gene promoter methylation is a common epigenetic change, involving extensive methylation at the 5-position of cytosine (C) in CpG islands within the promoter region and often extending into exons of regulatory genes.^{136,137} Generally, hypermethylation of CpG islands in promoter regions is associated with gene silencing, and hypomethylation is associated with gene activation. Both processes can be involved in a variety of functions including inactivation of tumor suppressor genes^{136,137} and modulation of cellular sensitivity to genotoxic compounds, which occurs via influences on the activity of genes involved in the metabolism of xenobiotics (substances foreign to the body).¹³⁸⁻¹⁴⁰ In lung cancer, more than 750 genes are inactivated by gene promoter hypermethylation, and additional genes are still being identified through genome-wide screening approaches.^{141,142} Additionally, smoking leads to hypomethylation of the aryl hydrocarbon receptor repressor (*AhRR*) gene. This epigenetic modification has been suggested as an indicator

of long-term exposure to tobacco smoke based on the observation that hypomethylation correlates with current and former smoking status, cigarette consumption, cumulative smoking, smoking duration, and time since quitting smoking.¹⁴³ *AhRR* hypomethylation has also been associated with decreased lung function and increased respiratory and cardiovascular morbidity.¹⁴³ However, it remains unclear what proportion of these epigenetic changes directly influence carcinogenic processes.^{136,137,142}

Epigenetic processes involving methylation may be partially dependent on one-carbon metabolism. One-carbon metabolism is a series of metabolic pathways, including methionine and folate cycles, that provide one-carbon or methyl groups for a variety of cellular functions.¹⁴⁴ Alcohol can interfere with one-carbon metabolism in several ways including by disruption of folate status and consequent inhibition of DNA methyltransferase,¹⁴⁵ thereby potentially generating aberrant DNA methylation patterns.¹⁴⁶ Additionally, alcohol consumption has been associated with variations in histone acetylation and histone methylation during carcinogenesis.¹⁴⁷ The hypermethylation of promoter genes with tumor suppressive characteristics has been observed in healthy tissue adjacent to tumors and oral premalignant lesions, suggesting that these epigenetic modifications are early events in oral carcinogenesis.^{148,149} In this context, tobacco use has been associated with several epigenetic changes in oral carcinoma that have also been observed as a consequence of alcohol exposure. Specifically, hypermethylation of the *CDKN2a* (*p16*), *DAPK*, and *CDH1* genes; inactivation of *P15* by promoter hypermethylation; and alteration of methylation patterns of the repetitive element long interspersed nuclear element-1 (LINE-1) have all been observed for both exposures. These modifications are believed to play a role in processes regulating cancer progression.¹⁴⁷

Effects of Smoking and Alcohol Cessation on Epigenetic Changes

Genome-wide methylation profiling studies have shown that smoking-induced epigenetic changes are dynamic and partially reversible. A study conducted by Guida and colleagues (2015)¹⁵⁰ showed that two distinct classes of CpG sites were affected by smoking cessation. Within decades after smoking cessation, methylation at some sites reverted to levels typical of women who have never smoked, while other sites remained differentially methylated, even more than 35 years after smoking cessation.¹⁵⁰ Another study identified both site-specific and regional changes in DNA methylation in women who smoked.¹⁵¹ The study also found substantial reversibility of methylation changes following smoking cessation, although some specific genes remained differentially methylated up to 22 years after smoking cessation. Additional research is needed to determine the extent to which reversible changes in methylation contribute to reduced risk of cancer after smoking cessation.

Few studies have investigated the effects of alcohol cessation on alcohol-induced epigenetic modifications. A study conducted among men undergoing treatment for AUD examined levels of gene-specific methylation (*ALDH2* and methylenetetrahydrofolate reductase [*MTHFR*]) and global methylation in DNA from peripheral blood leukocytes. Compared with healthy control participants, participants with AUD had higher gene-specific methylation and lower global DNA methylation; no statistically significant change in these methylation patterns was observed 3 months after the participants with AUD quit drinking.¹⁵² Additionally, a genome-wide

methylation analysis was conducted among men with AUD ($n = 99$) undergoing medically supervised withdrawal to identify methylation patterns associated with withdrawal from alcohol. This study identified a higher number of differentially methylated CpG sites in men with AUD than in control participants, a difference that decreased after 2 weeks of recovery from acute alcohol withdrawal.¹⁵³ More research is needed to determine how methylation and other epigenetic changes respond to changes in alcohol intake and whether these changes can be used as biomarkers of behavior or effect (see chapter 4).¹⁵⁴

Tumor Promotion

In addition to mechanisms in which tobacco and alcohol share the ability to promote similar molecular steps associated with tumor initiation, tobacco and alcohol act independently as tumor promoters.^{35,155} Tobacco use contributes to tumor promotion via influences on cell proliferation, cell cycle progression, and induction of nicotine.¹⁵⁵ Although the effects of alcohol have been studied less, its tumor promoting effects may involve effects on M2 macrophages¹⁵⁶ and histone deacetylation.¹⁵⁷

Tobacco and alcohol may also interact to influence tumor growth in tobacco-initiated cancer, and alcohol may promote tumor growth in cancers initiated by exposure to tobacco smoke. This observation is based on studies performed *in vitro* and in animal models.^{15,158} The tobacco-specific nitrosamines NNN and nicotine-derived nitrosamine ketone (NNK) are both categorized as Group I carcinogens (sufficient evidence for carcinogenicity in humans) by IARC,¹³ and NNN and NNK are major contributors to tobacco smoke carcinogenicity.¹⁵ A study conducted in rats found that ethanol caused an increase in the mean frequency and mean size of tumors induced by NNN or NNK in the rat esophagus, oral cavity, lung, and liver.¹⁵⁸ The study authors suggested that the ethanol-mediated promotion of NNN- and NNK-induced tumors may result from alcohol metabolism generating free radicals and increasing lipid peroxidation processes in the target tissue.¹⁵⁸ Many more mechanistic aspects of tumor promotion by co-use of tobacco and alcohol remain to be explored.

Genetic Susceptibility

Understanding how genetics influences cancer susceptibility in cancers related to tobacco and alcohol can help in understanding the mechanisms by which these environmental factors influence the carcinogenic process. The genome-wide association study (GWAS) approach has emerged as a common method for identifying genetic variants involved in cancer susceptibility, as well as related traits such as those that influence behaviors related to smoking and drinking (as described in chapter 6).¹⁵⁹ The GWAS approach assesses the common (>5%) genetic variation and low-frequency (1%–5%) genetic variation in the genome and thus identifies genetic variants without *a priori* evidence that a particular gene is involved.^{160,161} Large GWAS have been conducted for smoking- and alcohol-related behaviors and cancer types related to tobacco and/or alcohol use, such as lung, head and neck, and bladder tumors.^{162,163} As of 2026, the genes located near the genetic variants identified via agnostic GWAS include genes involved in smoking behaviors, alcohol metabolism, xenobiotic metabolism, and DNA repair,^{163,164} making them very similar to the genes and pathways associated with the mechanistic

evidence discussed previously in this chapter. As such, the agnostic GWAS approach supports much of the mechanistic data presented. The following sections discuss the genetic variants identified by GWAS and other approaches and how the tobacco- and alcohol-related carcinogenic mechanisms may be influenced by the functions of these genes.

Lung Cancer

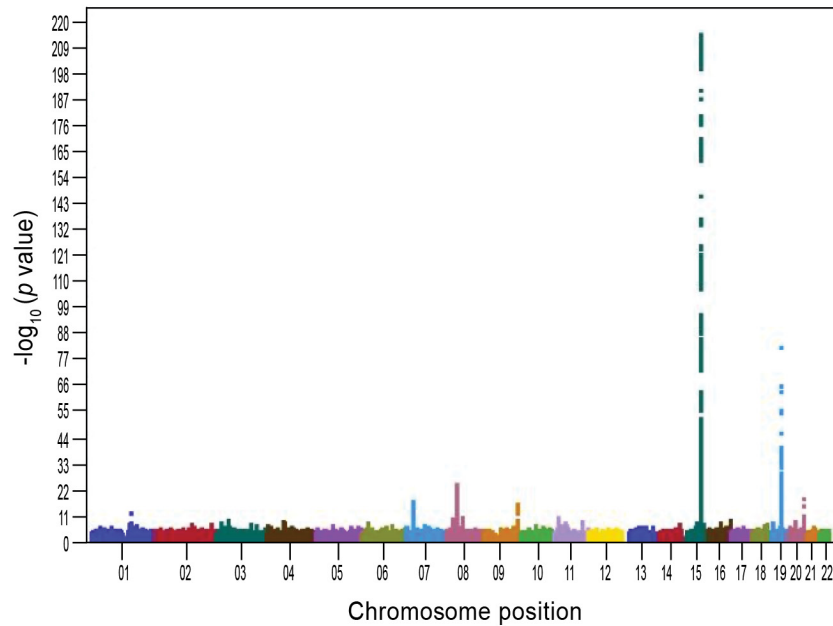
GWAS of lung cancer have identified loci involved in genetic susceptibility and identified genetic variants related to smoking behavior, DNA repair, telomerase activity, and immune response, as well as many variants where the target genes are unclear.¹⁶² Further, loci and variants related to alcohol and lung cancer have also been identified, including some that are related to alcohol metabolism and acetaldehyde.¹⁶⁴

Genetic Variants Related to Lung Cancer Risk and Smoking Behavior

One of the key insights stemming from genetic studies of lung cancer is that lung cancer genetic susceptibility tends to overlap with alleles associated with smoking behavior, as shown in Figure 5.3. First, key genetic variants identified from GWAS of lung cancer are the same as those independently identified from GWAS of smoking behaviors (e.g., in the genes *CHRNA5*, encoding the $\alpha 5$ nicotinic acetylcholine receptors (nAChR) subunit, and *CYP2A6*, encoding the enzyme that metabolizes nicotine); second, this genetic overlap extends to a polygenic risk model (i.e., many thousands of small effect susceptibility variants) estimated through sophisticated genetic analysis, such as genetic correlation based on linkage disequilibrium score regression.¹⁶⁵⁻¹⁷² This key finding is consistent with the idea that by influencing smoking behavior, such genetic variants modulate exposure to tobacco and, in turn, carcinogen exposure and lung cancer risk in susceptible individuals.^{165,168} The finding is further supported by the observation that the association with these alleles is predominantly observed in individuals with a history of smoking,^{165,168,173,174} and that there is more DNA damage, particularly mutations associated with tobacco exposure, in lung cancer tumors arising in genetically at-risk patients.¹⁶⁷ Gene and environment interactions involving tobacco use provide further evidence of the importance of preventing initiation of tobacco use to avoid development of lung cancer.

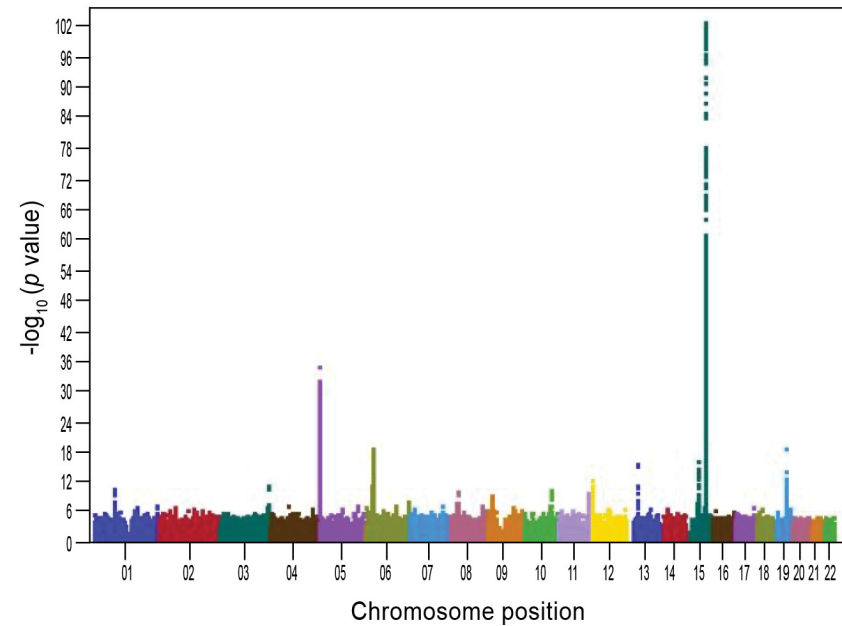
Figure 5.3 Manhattan Plots From Genome-Wide Association Studies of Cigarettes per Day and Lung Cancer in Participants of European Ancestry

Panel A: Cigarettes per Day



A: Genome-wide association study of cigarettes per day in European ancestry participants.¹⁷⁵

Panel B: Lung Cancer



B: Genome-wide association study of lung cancer in European ancestry participants.¹⁷³

Note. Each dot on the plot signifies a single nucleotide polymorphism (SNP). Genomic coordinates are displayed along the x-axis, and each color block represents a chromosome. The y axis is the negative logarithm of the association's p value for each SNP. The strongest associations have the smallest p values and their negative logarithms will be the greatest. Chromosomal region 15p25 encompasses *CHRNA5* and chromosomal region 19p encompasses *CYP2A6*.

Source: Plots created from data reported in Liu et al. 2019¹⁷⁵ and McKay et al. 2017.¹⁷³

The study of how these alleles individually influence the carcinogenic process provides insight into tobacco-related behavior and carcinogenesis. For example, susceptibility alleles influence different aspects of smoking behaviors, including smoking initiation, smoking intensity, smoking cessation, or combinations of these behavioral aspects of smoking.^{163,167,170,173,175}

Together, this genetic variation can help account for some of the variation in tobacco-related behaviors observed in human populations. Some alleles display rather paradoxically with respect to addictive behavior. For example, variants in the *CYP2A6* gene region (which plays a major role in nicotine metabolism) are associated with higher cigarette consumption, and higher risk of lung cancer and COPD, but an increased likelihood of successful smoking cessation.¹⁷⁶⁻¹⁷⁸

Other alleles also point to unexpected biological mechanisms. For example, the lung cancer susceptibility alleles near *CHRNA2* influence expression levels of this gene uniquely in the cerebellum, a brain area not typically linked with smoking behaviors.¹⁷³ It is worth noting that genetic variation in behaviors involving other forms of tobacco, such as the daily frequency of tobacco chewing, has also been documented.¹⁷⁹ These results suggest a complex set of genetic influences on behavior related to stages of nicotine dependence (see chapter 6).

Genetic Variants Related to Lung Cancer Risk That Are Unrelated to Smoking Behavior.

The GWAS approach has also identified many lung cancer susceptibility variants unrelated to smoking behavior.^{162,173} These genes are implicated in several hallmarks of cancer,¹⁸⁰ with genetic variants identified near genes involved in DNA repair, telomere maintenance, xenobiotic metabolism, and immune response, as well as other variants where the functional target unclear.^{162,173} Many of these alleles appear to be relevant to a particular histological subtype and smoking status. Examples of such loci include *TERT* (telomere reverse transcriptase, which helps maintain the end of telomeres),¹⁸¹⁻¹⁸³ *TP63* (tumor protein p63, which helps regulate cell activities including proliferation, cell maintenance, differentiation, cell adhesion, and apoptosis),^{184,185} *ROS1* (receptor tyrosine kinase, which acts as a cell surface receptor for a variety of growth factors),¹⁸⁶ *NRG1* (neuregulin 1, an epidermal growth factor),¹⁷³ and *ATM* (a tumor suppressor gene).¹⁸⁷ These loci are generally related to lung adenocarcinomas and imply smoking independent mechanisms of action. Other loci, such as variants in *BRCA2*¹⁸⁵ and *CDKN2A* (tumor suppressor genes),¹⁸⁸ *CHEK1*¹⁶⁷ and *CHEK2* (genes for regulating cell cycle checkpoints),^{185,189} *CYP1A1* (metabolism of xenobiotics),¹⁶⁷ and *IRF4* (regulatory transcription factor for immune cells)¹⁹⁰ appear particularly relevant to lung squamous cell carcinomas in people who use tobacco.

Elucidating the precise mechanisms by which these genetic variants confer susceptibility to lung cancer can help to better understand how tobacco influences the carcinogenic process and how genetic susceptibility may influence the legacy of tobacco-related damage in tissues in individuals who have quit tobacco use. Additionally, a better understanding of genetic susceptibility to tobacco-induced carcinogenesis could further inform efforts to identify how combined exposure to tobacco and alcohol influences carcinogenesis.

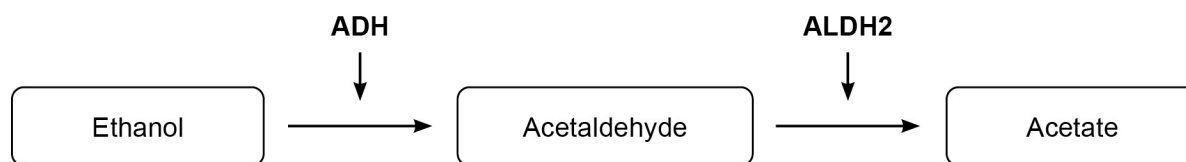
Aerodigestive Tract Cancers

Tobacco and alcohol are strong independent risk factors for many aerodigestive tract cancers, and substantial observational evidence supports a synergistic relationship between exposure to these two substances. As described in chapter 4, genetic variation in the alcohol metabolism gene *ALDH2*, specifically the *ALDH2**2 polymorphism, reduces the function of the *ALDH2* enzyme, leading to increased exposure to acetaldehyde in the oral cavity based on host and oral microbiome enzyme action. This increased exposure is associated with a higher risk of cancers of the head and neck and esophagus (squamous cell carcinoma).^{22,66,191,192} Genetic variation in various alcohol metabolism genes may increase or decrease the rate at which acetaldehyde is metabolized.^{192,193} Some of these variants lead to a relative increase in exposure to acetaldehyde in susceptible individuals, resulting in increased risk of cancers of the head and neck and esophagus.^{66,191}

The most studied *ALDH2* genetic polymorphism is rs671, a missense variant that results in the loss of function of the *ALDH2* protein and, as described in chapter 4, facial flushing. Individuals with *ALDH2* who drink alcohol also have increased cancer risk.^{191,194,195} As discussed in this chapter's section, "Acetaldehyde-Derived DNA Damage," research on *ALDH2* polymorphisms provides strong evidence for the role of aldehydes in alcohol-related head and neck cancers.^{67,194,195} Notably, the loss-of-function allele is also associated with a marked increase in the risk of developing esophageal squamous cell cancers, particularly in heterozygote carriers who consume alcohol despite experiencing facial flushing and other unpleasant effects.^{66,191,196} Genomic-based studies of the somatic environment of squamous cell esophageal tumors suggest a higher prevalence of somatic mutations associated with alcohol exposure (COSMIC signature SBS16) in tumors occurring in carriers than noncarriers.^{85,86} These findings are consistent with the hypothesis that the increased risk of esophageal cancer in *ALDH2* heterozygote allele carriers is mediated by acetaldehyde. Together, these observations support the need for alcohol-related cancer prevention efforts for people who are *ALDH2* variant carriers.⁶⁶

In addition to the *ALDH2* gene, studies have identified genetic variants in *ADH* genes^{197,198} related to alcohol consumption and risk of aerodigestive cancers, including evidence for interactions between these genetic variants and alcohol consumption (see Figure 5.4).¹⁹⁶ The *ADH1B* and *ADH1C* variants accelerate the metabolism of alcohol to acetaldehyde, elevating acetaldehyde levels in people with these variants who drink alcohol and increasing their risk of cancer.¹⁹⁹ Relatedly, studies in Japan and China have shown that, among people who consume alcohol, those who have less active *ADH* alleles are at greater risk for head and neck cancers,²⁰⁰ but in Western countries, where different polymorphisms are more common, mixed results are observed in relation to *ADH* activity and cancer risk.²⁰¹ Alleles of *ADH* and *ALDH2* that are present interact to modulate the levels of acetaldehyde associated with alcohol consumption, influencing both cancer risk and the effects of alcohol flushing on consumption.^{66,198,200}

Although research has identified the alleles and mechanisms through which the *ADH* variants influence cancer risk, the expression of *ADH* in tissues such as those of the lower gastrointestinal tract and subcutaneous adipose tissue, regions not considered primary for alcohol absorption and metabolism, as well as cardiovascular and lung tissues, suggests that the role of these *ADH* variants is not yet fully understood.⁶³

Figure 5.4 Simplified Diagram of Ethanol Metabolism

Note. Alcohol dehydrogenase (ADH) is the predominant enzyme catalyzing the conversion of ethanol to acetaldehyde, and aldehyde dehydrogenase 2 (ALDH2) metabolizes acetaldehyde into acetate. Alleles of *ADH* and *ALDH2* interact to influence the concentration of acetaldehyde in the aerodigestive tract, increasing or decreasing cancer risk.

Source: Seitz and Becker 2007.⁷³

There is overlap between lung cancer susceptibility alleles and aerodigestive tract cancers, particularly with regard to genetic variants located near DNA repair genes such as *RAD52*, *MDM4*, *BRCA2*, and *CHEK2*,²⁰²⁻²⁰⁶ suggesting the importance of the modulation of DNA repair in the context of the overlapping etiology of exposure to tobacco and alcohol. Mendelian randomization studies also support the causal relationship between tobacco and alcohol and head and neck cancers.²⁰⁷ One study reported that the susceptibility variants located near the *CHRNA5/3/b4* gene cluster associated with smoking propensity were related to aerodigestive tract cancers in women but not in men, although sex differences in the effects of these variants on smoking behavior were not observed.²⁰⁸ Additional research is needed to understand the biological basis for these findings.

Interactions Between Nicotine and Alcohol

Nicotine, the major alkaloid in tobacco, is responsible for the addictive properties of tobacco products.⁹ By driving smoking behavior, nicotine is responsible for the exposure to tobacco-derived carcinogens in people who use tobacco products. The mechanisms by which nicotine induces addiction to tobacco products are covered in chapter 6. The Surgeon General has concluded that “there is insufficient data to conclude that nicotine causes or contributes to cancer in humans,” but notes that “nicotine is bioactive for a number of carcinogenic mechanisms in experimental systems.”¹

Nicotine tolerance is higher in people with AUDs than in people without AUDs.^{209,210} People who smoke and have a history of heavy alcohol consumption score higher on measurements of nicotine reinforcement than those who smoke but have no history of AUD.²¹¹ Individuals who smoke have a higher prevalence of alcohol use than those who have never smoked (see chapter 2). Cross-tolerance (when repeated administration of one substance induces decreased response to another substance) between nicotine and ethanol consumption has been widely observed in animal models and also occurs in humans, although the evidence for such cross-tolerance in humans is somewhat indirect.²¹²⁻²¹⁴ This topic is also discussed in chapters 6 and 7. Further research is needed to understand the neurophysiological and genetic basis of these patterns of cross-tolerance and associated patterns of co-use.

These associations of increased tobacco and alcohol consumption with co-use could be related to both environmental and genetic factors. However, metabolic cross-tolerance likely plays a role because nicotine and ethanol share similar CYP-mediated metabolic pathways—CYP2E1 and CYP2B1, respectively—and both substances can induce these enzymes.²¹⁵ This induction can lead to more rapid elimination of ethanol and nicotine, which can result in increased consumption. Additionally, alcohol can induce CYP2A6, and high levels of CYP2A6 are associated with nicotine exposure in animal studies.⁹⁸ nAChRs are also expressed in tissues outside of the brain,²¹⁶ so it is plausible that the genetic effects of the susceptibility alleles with nAChRs—and their response to nicotine—may influence other tissues. Metabolic cross-tolerance and resulting behavior patterns can make quitting smoking or reducing alcohol consumption more challenging (see chapter 7).^{217,218}

Potential Interactions of Electronic Cigarettes and Alcohol

E-cigarettes are battery-powered devices that generate aerosol by heating a liquid (e-liquid) that typically contains nicotine, dissolved in a mixture of propylene glycol and/or vegetable glycerin, and flavorings. The health effects of e-cigarettes are influenced by many factors, including constituents of the e-liquid, physical characteristics of the device, and chemical reactions related to the heating characteristics of the device.²¹⁹ Assessing the health effects of e-cigarettes is complicated by the diversity of e-cigarette devices and e-liquids, their relatively recent entry into the tobacco marketplace, and their frequent use with other tobacco products.²²⁰ E-cigarette aerosol contains markedly lower levels of many of the harmful constituents and carcinogens present in cigarette smoke.²²¹ However, heating the main ingredients and the flavorings often used in the e-liquids results in exposure to many different carbonyl compounds including aldehydes, such as acetaldehyde and formaldehyde,²²¹ that are genotoxic and mutagenic.²²²⁻²²⁴ Although a detailed review of the potential short- and long-term toxicity of e-cigarettes is beyond the scope of this monograph, potential health effects have been documented for respiratory diseases²²⁵⁻²³¹ and cardiovascular disease.^{232,233}

A joint statement published by the American Association for Cancer Research (AACR) and the American Society of Clinical Oncology (ASCO) in 2022 described evidence linking e-cigarette use to cancer.²³⁴ The statement summarized research linking e-cigarette use to DNA damage, inflammation, and cell replication:

A growing body of evidence points toward a biologically plausible role for ENDS [e-cigarette] use in contributing to human carcinogenesis, based on the presence of carcinogens in ENDS aerosols; metabolites of carcinogens in human urine samples; inflammation markers in human lung swabs and blood samples; and cell culture and mouse experiment exhibiting DNA damage and inflammation.

Given these concerns, there is an urgent need for additional epidemiologic and mechanistic studies.

At present, there is also limited information about patterns of co-use of e-cigarettes and alcohol. However, like other tobacco products, most e-cigarettes contain nicotine. Alcohol consumption is likely to influence the use of e-cigarettes, and vice versa, given the potential increased tolerance and cross-tolerance between nicotine and ethanol, among other factors.

Additionally, the evidence suggesting a possible role for e-cigarettes to contribute to cancer—and the fact that both e-cigarette and alcohol exposure induce inflammatory processes and result in exposure to genotoxic compounds such as acetaldehyde—highlights the importance of studying the combined health effects of alcohol and e-cigarette use on cancer risk. Moreover, patterns of use and co-use of tobacco, nicotine, and alcohol products continue to evolve very rapidly. As other novel tobacco products (e.g., heated tobacco products, nicotine pouches) enter the marketplace, it will be important to study the influence of alcohol on patterns of their use across the life course and the combined health effects of co-use of tobacco products and alcohol, including the etiology, prevention, and treatment of cancer.

Chapter Summary

Tobacco smoke contains more than 7,000 chemicals, including at least 80 known carcinogens. Ethanol, the substance responsible for alcohol's addictive nature, is also the primary carcinogen present in alcoholic beverages. Alcohol may influence tobacco-related carcinogenesis by increasing exposure to carcinogens through its effects on membranes and metabolism, inflammation and oxidative stress, epigenetic changes, and promotion of tumor growth. Tobacco and alcohol co-use results in overlapping and distinct exposures to carcinogens, as well as effects on metabolic processes resulting in increased use. In addition, individual vulnerability to tobacco and alcohol carcinogens and the effects of nicotine and ethanol metabolites are influenced by genetic variation at multiple sites.

Research suggests that the interactive effects of combined tobacco and alcohol use on carcinogenesis of the aerodigestive system is largely associated with increased exposure to acetaldehyde, a potent carcinogen and the primary metabolite of ethanol. Other mechanisms include the alcohol-driven increase in the absorption of tobacco-related carcinogens, chronic inflammation, and the increased production of reactive oxygen species that both risk behaviors induce. Additionally, ethanol acts to increase membrane permeability in the aerodigestive system and acts as a solvent for tobacco-associated carcinogens, thus increasing their absorption. Together these processes increase uptake of acetaldehyde associated with both tobacco and alcohol use, as well as thousands of additional chemical compounds associated with tobacco use, including many carcinogens.

The oral microbiome plays an important role in metabolizing ethanol into acetaldehyde, and salivary acetaldehyde levels increase substantially with concurrent tobacco smoking and alcohol consumption. The interactive effects of tobacco and alcohol on aerodigestive tract cancers may be attributed to substantially increased acetaldehyde exposure from oral alcohol metabolism, combined with the high levels of acetaldehyde in tobacco smoke.

Further evidence for the causal role of acetaldehyde in alcohol-induced cancer is derived from studies of people with genetic polymorphisms that reduce the activity of aldehyde dehydrogenase 2 (ALDH2). Ethanol is converted to acetaldehyde in the oral cavity, but reduced ALDH2 activity in heterozygous individuals results in greater persistence of acetaldehyde. ALDH2-deficient individuals experience facial flushing and other adverse symptoms if they consume alcohol and are also at much higher risk of esophageal squamous cell carcinoma (among people completely lacking ALDH2 activity, the flushing response is so unpleasant that alcohol consumption is very low or absent, and consequently, the risk of esophageal cancer is much lower). Similarly, studies

of patients with Fanconi anemia, a rare genetic disorder characterized by an inability to repair damage to DNA caused by endogenous and exogenous carcinogenic aldehydes (e.g., acetaldehyde) have been informative. People with Fanconi anemia are at extremely high risk for developing certain types of cancer, including head and neck squamous cell cancers. These findings highlight that studies of people with rare genetic variants can provide important insight into carcinogenic mechanisms associated with tobacco and alcohol use.

Alcohol induces CYP2E1 which acts largely in the liver to metabolize many compounds, including the conversion of ethanol to acetaldehyde and then acetate. Additionally, CYP2E1 activates some carcinogens and produces reactive oxygen species, further pathways to carcinogenesis. Some studies have also examined potential interactions between tobacco and alcohol exposure and inflammation, retinol pathways, and other metabolic perturbations. Additionally, multiple studies in rodents report that ethanol can accelerate progression of NNN and NNK-induced tumors. The use of different types of tobacco products results in exposure to an overlapping, but not identical, mix of carcinogens as compared to those associated with cigarette smoking. How these different mixes of carcinogens interact with ethanol exposure is not well understood. For example, little is known about the potential interaction of e-cigarettes with alcohol on carcinogenesis. As new tobacco products enter the marketplace, it will be important to assess these interactions.

Tobacco cessation dramatically reduces risk of tobacco-related cancers but less is known about the effects of alcohol cessation or reduction and cancer risk. A 2024 IARC review found that the evidence is sufficient to indicate that alcohol use cessation or reduction reduces the risk of cancer of the oral cavity and esophagus, but the evidence is limited or inadequate for alcohol-caused cancer at other sites. Mechanistic studies suggest that these reductions in risk are related to reduced exposure to acetaldehyde, reduction in DNA damage, and reduced intestinal permeability, however more research is needed.

Research Needs

The study of the interactions of tobacco and alcohol on carcinogenesis from a mechanistic point of view is limited, especially when compared to the amount of similar research on tobacco and alcohol individually. This is a significant research gap because co-use of tobacco and alcohol is very common. Relatively few mechanistic or genetic studies have assessed detailed physiological and metabolic variation in the combined effects of tobacco and alcohol use over time, and even fewer have examined these processes after cessation of tobacco and/or alcohol use. The relative paucity of mechanistic and genetic studies in this context highlights the need to better understand the combined effects of tobacco and alcohol on carcinogenesis. Research is needed to assess how changes in tobacco and alcohol use, separately and especially together, influence inflammation, other carcinogenic processes, and cancer risk.

More research is needed on the combined effects of tobacco and alcohol on inflammation and oxidative stress. Both tobacco and alcohol use lead to production of reactive oxygen species and inflammation in multiple tissues.²³⁵⁻²³⁷ Less research has addressed the effect of heavy alcohol consumption on the response to proinflammatory agents in people who smoke and people who do not smoke, and results have been contradictory. More research on the combined effects of tobacco and alcohol exposure on oxidative stress and inflammation could clarify these mixed results.²³⁸

Evidence is limited or inadequate for understanding the effects of reduced alcohol consumption at multiple sites, with and without tobacco co-use. More research is needed to develop studies that have the power to observe a reduced risk, examine effects of reduction in alcohol consumption, and assess patterns of consumption over the life course, including consumption alongside tobacco use. Such studies might also provide opportunities to explore specific biologic mechanisms influencing cancer risk after different durations of cessation or reduced consumption of alcohol (and cessation or reduction alongside co-use with tobacco).³⁸

More research is needed on mechanistic aspects of combined tobacco and alcohol use and carcinogenesis covering diverse tobacco products and diverse populations. For example, relatively little research has examined the potential mechanisms of cancer risk among people who co-use e-cigarettes and alcohol.

More research is also needed on interactions between tobacco and alcohol use at low and moderate alcohol consumption levels. Most studies have focused on people who engage in risky drinking or those with alcohol use disorder. Few studies have examined the physiological effects of cessation or reduction in drinking at lower levels of consumption with or without concurrent tobacco use. Epidemiological studies show that even low levels of drinking increase the risk of cancer at multiple sites including female breast and colorectal cancer²⁴ and can increase the risk of liver disease.²³⁹ Results from human and animal studies also demonstrate a variety of physiological effects resulting from lower levels of alcohol consumption. However, most of the mechanistic studies on the effects of alcohol cessation include individuals with alcohol use disorder who are entering treatment programs and measure variables related to the overall health of the participants. Research involving this population can be challenging because of frequent loss to follow-up and the prevalence of multiple substance use disorders. More research is needed to explore the interactions of alcohol consumption at lower levels than for people with alcohol use disorder with diverse aspects of tobacco initiation, continued use, cessation, and relapse.

Advances in study design are needed to improve studies of co-use of tobacco and alcohol. There is a need for research using study designs that specifically address the effects of tobacco and alcohol exposures combined. These may include time-course designs, analysis of the effects after cessation of the exposures, dose-response designs to investigate effects considering lower levels of exposures, and investigation of effects in different populations. Research is also needed to specifically understand the mechanisms by which the initiation and/or promotion of cancer is influenced by smoking cessation and drinking cessation or reduction. Future research could evaluate how cessation or reduced use of tobacco and alcohol may result in metabolic changes, inflammation change, repair of DNA damage, and changes in risk of cancer.

Conclusions

1. **Tobacco and alcohol are known human carcinogens and increase the risk of cancer via both shared and independent pathways.** These pathways include exposure to dozens of carcinogens in tobacco, many associated with combustion, the presence of acetaldehyde in both tobacco smoke and as a metabolic product of ethanol, and physiological effects of ethanol on membrane permeability, hormonal and inflammatory pathways, and nutrient status.
2. **Combined carcinogenic and physiological effects of tobacco and alcohol provide a mechanistic basis for the synergistic increases in risk for aerodigestive tract cancers observed for co-use of tobacco and alcohol.** These mechanistic factors include exposure to acetaldehyde from both tobacco and alcohol; the composition of the oral microbiome, which results in increased acetaldehyde production; and increased permeability of aerodigestive tract membranes resulting in elevated uptake of tobacco carcinogens produced by tobacco combustion. Genetic variants of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase 2 (ALDH2) interact to influence cancer risk. These enzymatic variants influence acetaldehyde levels in the aerodigestive tract by influencing metabolism of ethanol to acetaldehyde (ADH) and acetaldehyde to acetate (ALDH2).
3. **Individuals with the ALDH2 variant (rs671) have increased risk of cancer from alcohol consumption.** Worldwide, an estimated 540 million people (primarily people of East Asian descent) are heterozygous or homozygous for mutations that reduce ALDH2 function, resulting in elevated levels of acetaldehyde, alcohol flushing responses, and increased risk of cancer. Efforts to address alcohol use in these populations are important for cancer control.
4. **Tobacco cessation reduces the risk of many types of cancer, and alcohol reduction or cessation reduces the risk of head and neck cancer.** There is a dearth of studies on whether alcohol reduction or cessation reduces the risk of other types of cancer. The mechanisms of carcinogenesis of tobacco and alcohol co-use are best understood for acetaldehyde-related pathways, but less well understood for hormonal- and inflammation-related pathways. Nevertheless, this mechanistic evidence strongly supports efforts to address tobacco and alcohol use and co-use to improve cancer control.

Appendix 5A Literature Search Strategy

The literature cited and summarized in this chapter was identified from searches conducted in 2023 using Google Scholar and PubMed, with a focus on peer-reviewed publications.

Various combinations of the following keywords were used for the searches: tobacco, cigarette smoking, alcohol, alcohol consumption, ethanol, mechanisms, interactions, genetic susceptibility, genome-wide association studies, germline, addiction, metabolism, and carcinogenesis. The publications resulting from these searches were reviewed for relevant citations. Publications that cited relevant articles were also evaluated for relevance. Additional studies published after 2023 were incorporated into the chapter based on their relevance.

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