Tobacco Use Insights

REVIEW

Tobacco, Genetic Susceptibility and Lung Cancer

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Abstract: Exposure to tobacco smoke is an established risk factor for lung cancer, although a possible role for genetic susceptibility in the development of lung cancer has been inferred from familial clustering of the disease and segregation analysis. Findings of familial aggregation and statistical evidence for a major susceptibility gene have led to the search for high penetrant, rare, single genes and low penetrant, high frequency susceptibility genes for lung cancer. The relatively small number of linkage studies conducted to date, have identified potential lung cancer susceptibility loci on chromosomes 6q, 12p, and 19q. A variety of studies have examined single nucleotide polymorphisms of several low penetrant, high frequency genes encoding for enzymes involved in the metabolism of carcinogens and DNA damage repair, as likely candidate susceptibility genes. These studies have produced somewhat conflicting findings and, when significant, only modest associations have been reported. Relatively few studies have looked for potential gene-environment interactions, explored associations between two or more genetic polymorphisms or evaluated interactions between genetic polymorphisms and endogenous risk factors. Few large scale genome wide association studies conducted recently have provided evidence that common variation on chromosome 15q25.1, 5p15.33 and 6p21.33 influences lung cancer risk and cancer types with strong environmental risk factors. It is hoped that newer research strategies, selecting candidate genes within pathways and genotype at multiple markers within a gene, employing new technologies, may allow complete coverage of the variation within candidate genes in multiple pathways and to unravel the genetic susceptibility to lung cancer. This knowledge could, in turn, be used to identify persons at risk, to individualize treatments such as chemoprevention, to personalize harms of smoking and to motivate cessation.

Keywords: lung cancer, tobacco smoke, cessation, genetic susceptibility, candidate genes

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Introduction

With over one million cases annually, Lung cancer is the leading and most common cancer in the world.[1] In 2002, 1.35 million new cases of lung cancer were diagnosed, representing more than 12 percent of all new cancer cases. Lung cancer is also the common cause of cancer deaths world wide, with 1.18 million deaths, accounting for 17.6 percent of the world total.[2] This deadly disease has a low 5 year survival rate of less than 10%–15% after initial diagnosis. The reasons mainly attributable for this poor survival rate are non-symptomatic features during early phase and late diagnosis of the disease. The prognosis of lung cancer patients is strongly dependent on how advanced their disease is. The 5 year survival rates for lung cancer patients in stage I, where the tumour has not spread, are about 70% whereas the survival is about 1% in stage IV where the disease has metastasized to other parts of the body. And a survival over 5 years is only about 10% even for patients with locally advanced tumours. This warrants the need to undertake every effort to diagnose lung cancer early in the course of the disease.

Risk Factors for Lung Cancer

Epidemiological studies have viewed lung cancer as a multifactorial disease. Smoking and occupational exposure to potential carcinogenic compounds are considered as major risk factors for lung cancer.[3] A number of occupational exposures such as aluminium production, arsenic, asbestos, bis-chloromethyl ether, beryllium, cadmium, hexavalent chromium, coke and coal gasification fumes, diesel exhaust, crystalline silica, nickel, radon and soot are established or suspected risk factors for lung cancer.[4] It is well accepted that 85% to 90% of all lung cancers are attributable to tobacco smoking[5] and it is estimated that tobacco smoking kills over 1,000,000 people each year, by causing lung cancer as well as other neoplasms. Smoking and lung cancer incidence shows a clear dose response relationship.[6] Small cell lung cancer (SCLC) is the form most attributable to smoking, squamous cell carcinoma is the next most attributable, and adenocarcinoma is the form least attributable to smoking. Passive exposure to environmental tobacco smoke causes additional cases, and exposure during childhood is more strongly associated with development of lung cancer than exposure as an adult. Several epidemiological studies have indicated that for a given number of cigarettes smoked, females may be at higher risk of lung cancer compared with males.[7]

Lung Cancer in Women

Even though lung cancer was once considered a disease of older men, today lung cancer is emerging as the significant cause of cancer deaths among women too. In the early 1900s lung cancer was reported to be rare in women but since 1960s, it has progressively reached epidemic proportions, becoming the leading cause of cancer deaths among women in the US. In US, women's smoking rates peaked in the 1960s and have been falling since then. When smoking in men has declined by half since the 1960s, smoking in women had only decreased by 25 percent. About 20 to 25 percent of US women continue to smoke. Worldwide, as women are given social and political freedoms, they pick up smoking. Observational studies have shown that women smoke differently than men do, for women it is a quick cigarette. They inhale more deeply and more quickly, so they may be prone to a different carcinogen exposure than men, because if one smokes really deeply, it will affect more distal airways which are farther from the major airways. Men usually have a slower, more lingering approach.[7,8] Starting in the 1980s, a vast number of women were diagnosed with lung cancer. There has been a two percent increase in death rates from lung cancer in women since 1930. Several case control studies seem to suggest that women may have an increased susceptibility to tobacco carcinogens. Results support a growing awareness that smoking presents greater risks to women than men. Early research indicates that susceptibility to tobacco smoke, estrogen and even difference in DNA may all play a role in the way lung cancer behaves in women.[8] Understanding what makes lung cancer in women unique should help researchers develop targeted therapies for women.

Familial Aggregation of Lung Cancer—Another Risk Factor

Although the major risk factor for lung cancer is cigarette smoking, it is also true that only 15% of life time smokers get lung cancer. This indicates that other factors which may affect the role of cigarette smoking are also important in the development of lung cancer. In addition to life style and environmental causes

(for e.g. smoking and radon exposure), the genetic constitution of an individual also has an important role in lung cancer predisposition or protection from it.[9–11] A possible role for genetic susceptibility in the development of lung cancer has been inferred from familial clustering of the disease. It has been known for more than 40 years that family history is also an independent risk factor for lung cancer, in addition to tobacco smoking, The first report on familial aggregation of lung cancer appeared as early as in 1963.[12] Actually, the importance of family history and genetic susceptibility to lung cancer has often been overlooked mainly because cigarette smoking is such an overwhelming and preventable risk factor. Most of the earlier epidemiological researchers believed that the similar smoking environment within the family setting or workplace accounted for those findings of aggregation of lung cancer cases in families. The presence of family history of lung cancer in first degree relatives had been reported to confer an excess risk of 30% and the familial aggregation was found to be stronger in the subset of patients with adenocarcinoma of the lung.[13] This study also reported that nonsmokers with lung cancer were 40% more likely than nonsmoking controls to report a first degree relative with lung cancer. Women were more likely than men to report such a family history. Based on a screening program of over 26,000 lung cancer patients, Bailey Wilson and colleagues[14] reported that 13.7% of the lung cancer patients screened, had at least one first degree relative with lung cancer. Later studies clarified that even after adjusting for smoking patterns, individuals with a family history of lung cancer were at approximately two- to threefold increased risk of developing this disease.[15–17] Available evidence supports the view that family history of lung cancer is associated with increased risk for lung cancer in both smokers and nonsmokers. The risk was higher if the relative was a woman. All these findings suggested a genetic predisposition to lung cancer after taking into account the familial clustering of smoking habits, family size and age structure. The findings of a stronger aggregation when the onset of disease is at an early age are indicative of an inherited component to risk. Consistent epidemiologic data and results of lung cancer linkage studies have suggested that family history should also be included as a high risk factor for lung cancer.

Early Onset of Lung Cancer in Non Smokers in Families

Exposure to environmental tobacco smoke (ETS) is considered to be a major lung cancer risk factor for non smokers. Young age at diagnosis often suggests an underlying genetic contribution to risk and several studies have shown that family history of an early onset lung cancer is associated with increased risk of lung cancer among never smoking relatives. Schwartz and colleagues[18] reported a six fold increased risk of lung cancer among relatives of non smokers with lung cancer diagnosed between the ages 40 and 59 years (95% CI 1.1–33.4) whereas Kreuzer and colleagues[19] found a non significant 3 fold increase in risk of lung cancer in female non smokers under age 46 years with a family history (OR 3.28, (95% CI 0.71–15.1)). A recent meta-analysis including 11 studies, evaluated risk associated family history of lung cancer among non smokers and reported that family history contributed to risk (RR = 1.51 (95% CI, 1.11–2.06)).[20] In six of these studies, with information on number of relatives affected, lung cancer risk in non smokers was increased 57% (95% CI 1.34–1.84) when one relative was affected and 2.5 fold when two or more relatives were affected (95% CI 1.72–3.70). The association between familial history of lung cancer and lung cancer in non smokers was reported[21] to be stronger in patients younger than 60 years at presentation and patients with adenocarcinoma. However, several factors such as incomplete or non adjustment for family structure and smoking among relatives, lack of validation of family histories, small sample size and lack of smoking status of all affected relatives are cited[22] as limitations relevant to the interpretation of these studies.

Genetic Susceptibility to Lung Cancer—Candidate Mechanisms

Genetic susceptibility of lung cancer had been examined in a number of epidemiologic studies. It is estimated that about 90% of lung cancers occur among ever cigarette smokers. However it is also true that most smokers live lung cancer free until a late age and that conversely, some moderate and non smokers develop the disease. This apparent paradox is, at least explained by variable genetic susceptibility to tobacco carcinogens.[23] People may differ in their susceptibility or resistance to tobacco carcinogens.

Particular individuals may be more susceptible to cigarette smoke. There is substantial inter-individual variation in the activity of enzymes that metabolize environmental agents, and maintain cell cycle control and immune function. These observations have given rise to the hypothesis that the carcinogenic potential of environmental (and endogenous) agents may be modified by common genetic polymorphisms in the genes that encode these enzymes.[24] Another explanation for inter individual susceptibility is varying cellular capacity to repair the genetic damage from tobacco carcinogenic exposures. Molecular epidemiologic studies have shown that sensitivity to mutagens and suboptimal DNA repair capacity (DRC) are independent risk factors for developing lung cancer.[25,26] These assays use a chemical or physical mutagen challenge (such as mutagen sensitivity assay) or measure cellular ability to remove adducts from plasmids transfected into lymphocyte cultures in vitro by expression of damaged reporter genes (host cell reactivation assay). This latter assay is a direct measure of repair kinetics whereas the cytogenetic assays indirectly infer DRC from cellular damage remaining after mutagenic exposure and recovery and as such likely reflect general and nonspecific impairment of the DNA repair machinery. A dose response relationship has been demonstrated with increasing risk associated with higher levels of sensitivity or poorer repair capacity. Bleomycin sensitivity is a highly heritable trait, the heritability being estimated as 75%.[27,28] Gorlova et al[27] estimated the life time probability of lung cancer for high risk groups in different smoking categories (current, former, and non smokers) and genetic susceptibility profiles. The highest risk subgroup were current smokers who were sensitive to bleomycin (above the control third quartile) and at the same time exhibited suboptimal DRC (below the control median). This group had the highest life time probabilty of 38% of developing lung cancer and they constituted 1/8 of the total population of current smokers. High risk male current, former and non smokers had life time probabilities for developing lung cancer of 38, 21 and 5% respectively. Females had lower probabilities to develop lung cancer; 15, 8, and 1.5% for high risk, current, former and non smokers, respectively. Screening of high risk smokers (12.5% of all smokers) reduced overall mortality by 7% compared to 3% reduction if all smokers were screened. Analogous results were obtained for former and non smokers. Through mutagen sensitivity and host cell reactivation assays, Gorlova et al[28] demonstrated that genetic susceptibility constitutes an important factor in the selection of a high risk group for early lung cancer detection. Interactions between the environment and genetic risk factors are strongly implicated in the etiopathogenesis of lung cancer.

Candidate Gene Locus (Loci) for Lung Cancer Susceptibility

Once familial aggregation has been established for a disease, the next step need to be evaluation of the pattern of inheritance of genetic susceptibility using segregation analysis to determine whether there is statistical evidence for the inheritance of a Mendelian major gene. And if strong evidence supporting an inherited component to disease is observed, the next step would be to pursue the localization of the potential disease gene(s). Although few earlier segregation analyses of lung cancer have not provided strong evidence for cancer—predisposing mutations,[29] a study by Gauderman et al,[30] in 337 extended pedigrees provided evidence that a Mendelian gene is segregating in these families. The estimated frequency of the high risk allele was 2%, carriers were estimated to have a relative risk of lung cancer of 17.3, compared with non-carriers.[30] Two studies[31,32] have investigated whether there is statistical evidence for the inheritance of a major gene for lung cancer using segregation analysis. Both studies have reported that the pattern of lung cancer occurrence in families is consistent with mendelian codominant inheritance of a rare autosomal gene. Sellers et al[31,33] estimated that this putative gene is responsible for 69% of the lung cancer seen at age 50 years, 47% at age 60 years, and 22% at age 70 years. Results from these studies indicated that the pattern of lung cancer occurrence in families with lung cancer was consistent with Mendelian codominant inheritance for early age—at—onset of a rare autosomal gene.

Findings of familial aggregation and statistical evidence for a major gene have led to the search for high penetrant, rare, single genes for lung cancer susceptibility through linkage studies. Multipoint parametric linkage analysis conducted had provided evidence for a dominant or codominant rare gene for lung cancer.[17] The study by Greenberg and Abrieu[34] demonstrated the existence of a chromosomal region linked to lung

cancer inheritance in families and also provided some initial insight into the relationship between smoking and lung cancer occurrence in high risk families. One lung cancer family linkage study, conducted by the Genetic Epidemiology of Lung Cancer Consortium is ongoing and the initial findings were reported by Bailey Wilson and Colleagues.[14] These results provided evidence supporting linkage between a region on chromosome 6q23–25 (146–164 cM) and lung, laryngeal and pharyngeal cancer. It was shown that lung cancer risk among putative carriers of the linkage region was increased even in non smokers. The region identified was large, encompassing 74 known genes and 41 unknown genes, including four putative tumour suppressor genes (SASH1, LATS1, IGF2R, and PARK2) as well as genes involved in regulating cellular proliferation and preventing DNA damage. In another alternative approach, using 13 microsatellite markers, Yanagitani and colleagues[35] reported increased risk of lung cancer associated with marker D12S0134, suggesting a putative lung adenocarcinoma locus in this region, (12p11–12) which falls between the location of Kras2 and Krag oncogenes. It is interesting to note that microsomal GSTM1, which is involved in detoxification of oxygen radicals and matrix Gla protein (a lung extracellular matrix component) is located on 12p and could also be a potential candidate gene for lung function. In a genome wide association study using 322 microsatellite markers, Yanagitani and colleagues[36] reported other significant associations of markers D6S474 (at 6q22) and D19S246 (at 19q13.3) with lung adenocarcinoma and suggested putative lung adenocarcinoma loci in these regions. Again a noteworthy observation is that DNA repair genes ERCC1 and ERCC2 are located in this region at 19q13.2–3. Further search for lung cancer susceptibility gene is ongoing.

Association with Other Diseases

Researchers have found that the genetic disorder alpha 1-antitrypsin deficiency (alpha 1 ATD) could explain up to about 12 percent of lung cancer patients[37,38.] Alpha 1-antitrypsin is an antiprotease that binds and inhibits neutrophil serine proteases such as elastase in the lung, protecting against lung tissue destruction. A normal alpha 1 ATD gene produces a protein that stops enzymes from breaking down elastin which keeps lung tissue elastic for normal function. Carriers of alpha 1 ATD commonly develop emphysema and/or chronic obstructive pulmonary disease (COPD). The WHO estimates that at least 10 million Americans and 120 million people world wide are alpha 1 ATD carriers. Yang et al[37] tested whether alpha 1 ATD carriers were predisposed to a higher risk of lung cancer and reported that all alpha 1 ATD carriers were at a similarly increased risk of developing lung cancer regardless of smoking status. Those who had never smoked were 2.2 fold higher risk; light smokers had a two fold greater risk, and moderate to heavy smokers had a 2.3 fold increased risk. Although there was no absolute definition, less than 20 pack years of smoking cigarettes was defined as light, more than 40 pack years as heavy. (A pack year is the number of packs of cigarettes smoked per day multiplied by the number of years the person has smoked). Increased risk of lung cancers among alpha 1 ATD carriers was independent of a family history of lung or other cancers. In a recent dual case control design, Yang et al[39] reported that alpha 1 ATD carriers had a 70% higher risk of developing lung cancer than non-carriers (OR = 1.7, 95% CI 1.2–2.4). Stratified analysis by tumour histologic subtypes showed a significant increase in adenocarcinoma and squamous cell carcinoma among alpha 1 ADT carriers. Although these studies help explain why people who have never smoked can develop lung cancer, it doesn't mean that people who don't have the gene won't develop lung cancer. Smoking remains the overwhelming risk factor for lung cancer development.

An excess risk of lung cancer among patients with COPD has been explained to be the result of lung tissue damage from emphysema or chronic infection or inflammation of the lungs or both.[4] Sun and Young[41] hypothesized that an imbalance between neutrophil elastase and α1-antitrypsin could contribute to development of lung cancer. The gene for α1- antitrypsin is located on chromosome 14q32.1 and over 75 alleles have been identified. Two common alleles (S and Z) are associated with α1-antitrypsin deficiency. Cigarette smoking contributes to faster tissue destruction through stimulation of neutrophils and increased secretion of elastase and further inactivation of α1-antitrypsin. Both Z and S allele carriers have been reported to be more common among patients with lung cancer than in the general population.[37]

Although a single gene for lung cancer has not yet been identified, occasionally lung cancer has been found to occur in families with Li-Fraumeni syndrome,

a condition that is associated with inherited mutations of the p53 gene.[42] This demands the need to accrue large, multi generation pedigrees with multiple affected family members for genome—wide searches for lung cancer genes. But there are limitations in pursuing a single gene for lung cancer. The fact that Lung cancer families are rare and occur in only 1% of the population, age of disease onset is usually in the mid to late 60s, affected relatives are typically deceased due to high fatality rate for lung cancer, smoking data which needs to be collected on all family members might not be available as many of whom might have deceased, are some to the confronting problems. In order to overcome these problems, collaborative efforts especially by the Genetic Epidemiology of Lung Cancer Consortium and few others are already underway.

Association Studies for Susceptibility Gene Identification

Alternate approaches such as association studies are also used to detect lung susceptibility on low penetrance genes that are common. There is substantial inter-individual variation in the activity of enzymes that metabolize environmental agents, repair DNA damage, and maintain cell cycle control and immune function. These observations have given rise to the hypothesis that the carcinogenic potential of endogenous and exogenous agents including tobacco smoke may be modified by common genetic polymorphisms in the genes that encode these enzymes.[24] Genetic polymorphisms are DNA sequence variations, mainly single nucleotide polymorphisms (SNPs) occurring with a frequency of more than 1% in the population.[43] Such polymorphisms usually will have a relatively low penetrance and may be silent in the absence of exposure. Genetic polymorphisms may result in defective protein function and stability, altered posttranscriptional processing, or altered levels of expression. Association studies in lung cancer have primarily focused mainly on polymorphisms in genes coding for enzymes involved in Phase I and Phase II metabolism of carcinogens in tobacco smoke and DNA damage repair.

Single Nucleotide Polymorphisms as Candidates for Lung Cancer Susceptibility

Multistage models of carcinogenesis propose that carcinogens derived from chemical components in tobacco smoke react with the DNA of respiratory epithelial cells.[44] In tobacco smoke, more than 60 lung carcinogens have been identified.[45] Pro-carcinogens in tobacco smoke must be metabolically transformed in order to exert their carcinogenic effect.[46] At the same time, other enzymes detoxify these chemicals into inactive compounds, thus creating a dynamic equilibrium of carcinogen concentration in lung tissues for any given level of tobacco smoke inhalation. Some of the carcinogens initiate mutagenic changes while others promote the growth of these mutated cells or disable genes that suppress tumour growth. All the carcinogenesis steps such as mutagenesis, growth promotion and inhibition of tumour suppression might be necessary for the development of clinically evident cases of lung cancer. This in a way should explain, the salutary effects of smoking cessation on lung cancer incidence even after years of tobacco use. Epidemiologic studies have demonstrated that low penetrance, high prevalence polymorphic phase I and phase II enzymes of the Cytochrome P 450 system may alter susceptibility to lung cancer.[47] Susceptibility to lung cancer is affected by existence of polymorphic genes controlling the levels of metabolic activation and detoxification of carcinogens. Genetic polymorphisms in the metabolism genes that cause carcinogens to accumulate, to a greater or lesser degree in any individual are highly suspected in contributing to the development of lung cancer. Many of these compounds are converted into reactive carcinogenic metabolites by Phase I enzymes and are removed by Phase II enzymes.[48] It has been reported[49] that in the lung, at least 57 Cytochrome P 450 enzymes are expressed, resulting in multiple species of reactive metabolites. The Phase II enzymes especially Glutathione S Transferases (GSTs) and N Acetyl Transferases (NATs) are responsible for removal of reactive metabolites[50,51]

Multiple genes have been studied to identify their genetic polymorphisms in the human population that confer differential susceptibility to carcinogens in cigarette smoke and for their potential associations with lung cancer risk. GST enzymes are known to catalyze detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins, and products of oxidative stress and are mainly involved in carrying out the conjugation of glutathione to electrophilic substances such as reactive intermediates from Polycyclic Aromatic Hydrocarbons (PAH).

GSTμ, GSTθ, GSTπ, all of which are polymorphic, are the most important enzymes in this pathway.

GSTM1 has been documented to be the gene most frequently associated with increased risk. An earlier review[52] of 12 case control studies concluded that GSTM1 deficiency is a moderate risk factor for all histological subtypes of lung cancer with OR of 1.41 (95% CI, 1.2–1.6). When these studies were stratified to race, an elevated OR was detected in Japanese population (OR = 1.60, 95% CI 1.3–2.1) but not in Caucasians (OR = 1.17, (95% CI, 0.98–1.40)). In an Indian population Sreeja et al[54] reported that the null genotypes of both GSTM1 and GSTT1 conferred an OR of 2.98 (95% CI = 0.984–9.024, P = 0. 053) for lung cancer susceptibility risk. In a meta analysis of 98 published genetic association studies investigating the relation between the GSTM1 null variant and lung cancer risk, which included altogether 19,638 lung cancer cases and 25,266 controls, GSTM1 null status was found to confer a significantly increased risk of lung cancer to East Asians (OR = 1.38 (95% CI, 1.24–1.55) where as such a genotype did not confer increased risk to Caucasians.[55] These results indicated that the role of GSTM1 deficiency in lung cancer susceptibility risk could vary in each ethnic population and so should be assessed differently.

CYP1A1, another principal enzyme involved in the conversion of PAH into cancer causing compounds has also been studied extensively for their lung cancer associated risk. The ratio between CYP1A1 and GST enzyme activities is a critical determinant of the target dose of carcinogenic Benzo(a) pyrene diol epoxide (BPDE) and other DNA reactive PAH metabolites. In the analysis combining polymorphisms and interactions with smoking, on lung cancer patients in India,[56] those patients who were smokers and having a GSTT1 null genotype showed increased risk association (OR = 2.24 (95% CI 1.020–4.929, P = 0.045)) after adjustment for age and gender. Patients who smoked and had the CYP1A1 variant genotype also showed higher risk association (OR = 2.947 (95% CI 1.090–7.968, P = 0.033)) compared to their non-smoking counter parts possessing wild type genotypes. The results from this study suggested a plausible combined role for these enzymes in lung cancer susceptibility. In a comprehensive study of 105 polymorphisms in 31 xenobiotic metabolizing enzymes,[57] Zienoldinny et al found few strong and robust associations. Several SNPs in the phase I genes CYP1B1, CYP2D6, CYP2E1, and CYP3A4 were found to be associated with risk of NSLC. Moreover, significant associations with multiple SNPs in Phase II genes ALDH2, COMT, EPHX1, SOD2, NAT1, NAT2, GSTM3, GSTP1, GSTT2 and MPO were also found. On multiple testing, three novel associations of NSLC risk with SNPs in the CYP1B1 (Arg 48 Gly), COMT (Val 58 Met) and GSTT2 (Met 139 Ile) genes were found noteworthy. However, only four of the previously reported associations with polymorphisms in the GSTP1 (Ala 14 Val), SOD2 (Val 16 Ala), EPHX1 (His 139 Arg) genes and the NAT1 fast acetylator phenotype remained significantly associated with lung cancer. Furthermore, in an Indian study by[54] Sreeja et al, other combination genotypes such as GSTM1 null and GSTP1 Ag/GG (OR = 2.1 (95% CI, 1.049–4.287, P = 0.03)); GSTT1 null and GSTP1 AA (OR = 2.5, (95% CI, 1.336–4.909, P = 0.005)); GSTT1 null and GSTP1 AG/GG (OR = 4.2 (95% CI, 1.017–8.767, P = 0.001)) emerged as predisposition genotypes associated with lung cancer susceptibility risk.

A number of studies have been conducted to evaluate the potential roles of the polymorphisms Ser 326 Cys of human 8-OxoG glycosylase 1 (hOGG1) and X ray repair cross complementary group I (XRCC1) genes in the risk of lung cancer. Sugimura et al[58] reported that the Cys/Cys polymorphic genotype of hOGG1 was associated with increased risk for lung cancer in Japanese (OR = 1.71, (95% CI 0.92–3.19)) especially squamous cell carcinoma (OR= 3. 01 (95% CI = 1.33–6.83)). Few studies have investigated the potential role of XRCC1 Arg 399 Gln and XPD Lys 751 Gln polymorphisms on lung cancer risk. These polymorphisms have been shown to have functional significance and also postulated to be responsible for the inter individual DNA damage repair variations in the general population and for a low DNA repair capacity phenotype characteristic of cancer patients.[25] Divine et al[59] reported that the Gln/Gln genotype was associated with an increased risk of adenocarcinoma and risk estimates for the risk genotype was much higher in non Hispanics whites than in Hispanics. Another hospital based study[60] found a further increased risk among squamous cell cases among Koreans (OR 3.3. (95% CI = 1.2–9.2)) and Sreeja et al[61] reported significantly higher ORs for XRCC1399 AA genotype (OR = 2.1 (95% CI 1.224–3.699, P = 0.007))

association with lung cancer susceptibility in an Indian population. The A751C variant of XPD has been associated with increased risk of lung cancer in several studies.[62–64] The carriers of XPD 751 AC genotype were at 2.7 fold (95% CI 1.12–6.93) higher risk for lung cancer than carriers of the AA genotype in northeastern Chinese population.[65] In Norwegian lung cancer population, Ziennolddiny et al[66] and in an Indian population Sreeja et al[61] also reported significant association of XPD heterozygous variants in modulating NSCLC risk.

Mutations in P53 gene, which are frequent in tobacco related cancers, is the most common genetic alteration in lung cancer, found in about 60% of the cases.[67] The mutational load is often higher in cancers from smokers than from nonsmokers. Benzo (a) pyrene diol epoxide (BPDE)–adducts are involved in the induction of p53 mutations and probably in the causation of human lung cancer associated with cigarette smoking. The codon 72 polymorphism in exon 4 of p53, which is carried by 20%–40% of the population polymorphism, has been postulated[68] to act as a risk factor in lung cancer, by altering the ability of p53 protein to induce apoptosis, influencing the behaviour of mutant p53, and decreasing the DNA repair capacity. The study by Alexandrov et al[69] were consistent with the hypothesis that BP (PAH) induce G: C to T: A transverse mutations in the hotspot codons of the P53 tumour suppressor gene and are thus involved in malignant transformation of the lung tissue of smokers. Fan et al[70] investigated the influence of polymorphic genotype TP53 Pro/Pro on lung cancer susceptibility in a Chilean population. The P53 Pro/Pro genotype was found to contribute significantly to lung cancer susceptibility risk (OR 3.88 (95% CI 1.16–13.39)). The P53 Pro/Pro genotype showed strongest association with squamous cell carcinoma in a study by Mechanic et al.[71] In a case control study involving 211 lung cancer cases and 211 controls, Sreeja et al[72] reported an OR of 2.5 (95% CI = 1.470–4.302, P = 0.001) for the p53 Pro/Pro variant genotype for lung cancer susceptibility, in an Indian population and the risk tended to be higher in women (OR = 2.4, P = 0.003).

In a large Caucasian population, involving a total of 1694 cases and controls, Miller et al[73] examined the association of combined variant genotypes or double variants (DVs) of three genes (GSTP, GSTM1 and P53 codon 72) and lung cancer risk compared with their corresponding "double wild type" genotypes. Individuals with the GSTP1 and GSTM1, DV (P1- M1 DV) had a marginally significant higher risk of lung cancer compared with their double –wild type counter parts (AOR 1.60 (95% CI 0'95–2.70)) and a significantly higher risk was found for the GSTP1, p53 DV (p1–p53 DV; AOR 1.99 (95% CI 1.12–3.53)). Among individuals aged 55 or younger, these risks were even higher; for the P1-M1 DV, the AOR was 4.03 (95% CI 1.47–11.1); for the P1-P53 DV, the AOR was 5.10 (95% CI 1.42–18.30). Genetic polymorphisms in cell cycle regulatory genes MDM2 and P53 have also been investigated for their contribution to the risk of developing lung cancer. In a molecular epidemiological study, Zhang et al[74] investigated the association between genetic variation in the promoter region of MDM2 and the coding region of TP 53 (Arg 72 Pro) and the risk of developing lung cancer. An increased lung cancer risk was associated with MDM2 GG (OR = 1.83 (95% CI 1.45–2.32)) and TG (OR = 1.33 (95% CI 1.09–1.63)) genotypes. Also an increased risk was associated with the TP 53 Pro/Pro genotype (OR = 1.47 (95% CI 1.17–1.85, P = 0.003)) compared to the Arg/Arg genotype. Furthermore, the gene-gene interaction of MDM2 and TP53 polymorphisms (MDM2 GG and P53 Pro/Pro genotypes) increased lung cancer risk in super-multiplicative manner (OR = 4.56 (95% CI 2.76–7.54)). Significant interactions were also observed between these polymorphisms and smoking (OR = 10.41 (95% CI 5.26–20.58)) for smokers with both the MDM2 GG and TP53 Pro/Pro genotypes, indicating further that both the polymorphisms interact with smoking.

Nitrosamine 4 (methylnitrosamino)-1 (3-pyridyl) -1-butanone (NNK) is one of the most potent and abundant carcinogen in tobacco and tobacco smoke.[75] NNK is efficiently converted to 4 (methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) by carbonyl reduction and glucuronidation is the major detoxification pathway for NNAL. The UDP—glucuronyltransferases (UGTs) family of genes mediates glucuronidation activity. Polymorphisms in the UGT2B17 and UGT1A4 genes, are associated with altered/lowered rates of NNAL glucuronidation.[76] In a case control study of 398 lung cancer patients and 697 community controls, the UGT2B17 deletion was associated with a significant increase in risk of lung adenocarcinoma

in women (OR = 2.0 (95% CI 1.01–4.0)) and not with any other histologic types of lung cancer.[77] The association of the UGT2B17 deletion with increased lung adenocarcinoma in women is consistent with its association with decreased NNAL glucuronidation rates in women. Dellinger et al[78] demonstrated that UGT1A10, which is an important detoxifier of PAH in tobacco smoke is expressed in human lung and suggested that the UGT1A10 codon 139 polymorphism could be an important determinant in risk for tobacco related cancers including lung cancer. Further larger studies could identify potentially combined high risk UGT genotypes and lung cancer risk.

The gene ELA2 which codes for neutrophil elastase is located on chromosome 19p13.3 and has been evaluated as a candidate in lung cancer etiology. Taniguchi and colleagues[79] identified 2 polymorphisms in the promoter (T–903 G) (REP–a) and G–741 A (REP–b) and evaluated the contribution to lung cancer risk from these polymorphisms, in a case control study. The TT genotype at REP–a was associated with a 2.3 fold increased risk of lung cancer (95% CI, 1.2–4.7) whereas the GG genotype at REP–b was associated with a 1.4 fold increased risk (95% CI, 1.0–2.0). Higher promoter activity was associated with the risk genotypes. For the TT genotype at REP–a, similar findings were reported by Park et al[80,81] (OR 3.2; 95% CI, 1.03–10.4).

Neutrophil elastase is involved not only in lung tissue breakdown, but also in activation of matrix metalloproteinases (MMPs) which are proteolytic enzymes that degrade extracellular membranes. An MMP1 promoter single nucleotide polymorphism G-1607 GG have been associated with lung function decline in smokers[82] This MMP1 promoter SNP and another MMP3 promoter SNP have been reported[83,84] to alter risks of lung cancer whereas Yu et al[85] observed a two fold increase (95% CI, 1.7–2.8) in lung cancer risk association for those with an MMP2 promoter SNP. Furthermore, the MMP genotype—lung cancer risk association was reported to be stronger as cigarette smoke exposure increased.[83,85]

Microsomal epoxide hydrolase which is found in bronchial epithelial cells, hydrolyses arenes, alkenes and aliphatic epoxides making them less reactive. It has been observed that polycyclic aromatic hydrocarbons found in tobacco smoke become more reactive when metabolized by microsomal epoxide hydrolase, in some instances. The gene EPHX1 coding for microsomal epoxide hydrolase has been mapped to 1q42.1 and includes two polymorphisms Try 113 His and His 139 Arg which are associated with decreased and increased activity respectively. Few studies have reported that the predicted high activity genotype is associated with increased risk for lung cancer.[85–87.]

Genome Wide Association Studies

Recently, high density arrays of SNP loci has been screened to produce profiles of germline alterations associated with increased lung cancer risk. Few large scale genomic analyses of lung cancers, including genome wide association studies characterizing genetic predisposition factors to lung cancer risk in case-control series have been published. Hung et al[88] conducted a genome wide association study of lung cancer comparing 310,023 SNPs between 1926 cases and 2522 controls. They identified a locus on chromosome region 15q25, an area that includes a cluster of nicotinic acetyl choline receptor gene that was strongly associated with lung cancer. An increased risk with the chromosome 15q25 locus and lung cancer in nonsmokers, as well as the lack of an association with smoking related head and neck cancers was observed. This indicated that the disease mechanism with lung cancer is unlikely to be explained by an association with tobacco addiction. Independent biological data also suggest that nicotinic acetyl choline receptors could be involved in lung cancer through other mechanisms. It has been suggested earlier that N-nitrosonornicotine and nitrosamines may facilitate neoplastic transformation by stimulating angiogenesis and tumour growth mediated through their interaction with nicotinic acetylcholine receptors.[89] The expression of these receptors can also be inhibited by nicotine receptors antagonists, which, if confirmed to be involved in disease etiology through such a mechanism, implies possible chemoprevention opportunities for lung cancer. For further confirmation and to identify additional lung cancer susceptibility variants, further analyses in multiple diverse populations will be required. The data from Hung's study[88] provide compelling evidence of a locus at 15q25 predisposing to lung cancer and reinforce interest in nicotinic acetylcholine receptors as potential disease candidates and chemo preventative targets. In order to identify further susceptibility gene loci, this group[90] carried out

another genome wide association study genotyping an additional 1291 cases and 1561 controls, for a total of 3259 cases of lung cancer and 4159 controls with genome wide data. They identified a new susceptibility locus for lung cancer that comprises two potential candidate genes: TERT (Telomerase Reverse Transcriptase), an essential component of telomerase production and of carcinogenesis, and CLPTM1L (Cleft Lip and Palate Transmembrane 1 like), which may induce apoptosis. But however, nature of the causative alleles remains unclear. Their results suggest that either one or both these genes may have role in lung cancer etiology.

To explore the impact of common variation on the risk of developing lung cancer, Broderick et al[91] conducted two phase genome wide association (GWA) studies. In Phase 1, they compared the genotypes of 511,919 tagging SNPs in 1952 cases and 1438 controls. In Phase 2, 30568 SNPs were genotyped in 2465 cases and 3005 controls. In the combined analysis of phases 1 and 2, the strongest associations identified were defined by SNPs mapping to 15q25.1, 5p15.33 and 6p21.33. Variation at 15q25.1 but not 5p15.33 or 6p21.33 was strongly associated with smoking behaviour with risk alleles correlated to higher consumption. The region 15q24–25.1 contains genes for nicotinic acetylcholine receptor subunits, indicating a possible association with nicotine dependence. Variation at 5p15.33 was shown to significantly influence induction of lung cancer histology. These genome wide association studies,[92,93] have provided evidence that common variation at 5p15.33 (location of TERT and CLPTM1L genes) and 6p21.33 and 15q25.1 (CHRNA5–CHRNA3) influences lung cancer risk and cancer types with strong environmental risk factors. Subsequent studies by Spitz et al[64] suggested that the 15q24–25.1 locus polymorphism is not associated with lung cancer in non smokers, supporting that its primary influence on lung cancer might be through an influence on nicotine addiction. To examine whether variations at these 3 locations influence the impact of environmental risk factors on lung carcinogenesis, Zienolddiny et al[66] studied the relationship between the DNA adducts in lung tissue adjacent to tumour from 204 lung cancer cases. The TERT—CLPTM1L locus was found to be associated with significantly higher levels of bulky aromatic/hydrophobic DNA adducts. These data demonstrated a potential association between the TERT—CLPTM1L variant and levels of bulky DNA adducts and hence a basis for susceptibility to the development of lung cancer. However, these genome-wide association studies have not defined genetic factors contributing to lung cancer risk in non smokers.

Even though hundreds of candidate gene association studies have been conducted for identification of lung cancer predisposition genotypes, the specific genes that are associated with alterations in risk remain poorly understood. Many have not considered the gene-gene and gene-environment interactions in their studies and some of the studies lacked large sample sizes too. In spite of having limitations including population heterogeneity due to significant differences in allelic frequencies between races and ethnicities, differing case and exposure definitions and differing genotyping methods, large consortia studies have the capacity to pool their findings across their studies and to increase their sample sizes and power. Furthermore, the limited number of candidate SNPs studied represent only some of the variation within a gene, may not be functional and unlikely to be acting alone. As there are relevant pathways yet to be fully characterized, newer approaches that select candidate genes within pathways and genotype at multiple markers within a gene, employing new technologies have been initiated worldwide. These may allow complete coverage of the variation within candidate genes in multiple pathways and to unravel the genetic susceptibility to lung cancer.

Use of Genetic Susceptibility Information in Smoking Prevention and Cessation

According to IARC studies,[94] people who quit smoking live longer than those who continue to smoke and smokers who quit before age 50 cut their risk of dying in the next 15 years in half compared to those who continue to smoke. With smoking cessation, the risk of lung cancer decreases over time. The amount of time it takes to reach the risk of a non smoker depends on the number of years smoking cessation as well as the number of cigarettes smoked per day. The more cigarettes smoked per day, the longer it takes to reach a level of risk close to that of a non smoker. Ex-smokers never reach the same low level of risk as a non-smoker. At each encounter with a healthcare provider,

all smokers should be encouraged to quit smoking. Strategies available to assist with cessation include nicotine replacement therapies, drug therapy, and psychological therapies that include social support in a group setting. A combination of strategies with the inclusion of psychological support has proven to be the most successful approach. Furthermore, knowledge of genetic susceptibility for lung cancer could be used to identify persons at risk and to individualize treatments, such as chemoprevention.[95]

Chemoprevention

Although avoidance of tobacco product is the surest way to decrease lung cancer risk, chemoprevention promises to be a useful adjunct strategy and has quite recently become an accepted standard of treatment for lung cancer. Chemoprevention involves the use of dietary or pharmaceutical agents to treat individuals identified as having high risk predisposition genotypes or premalignant lesions and to inhibit or to reverse the carcinogenic process respectively.[96] Genetic, molecular and phenotypic markers could be used to select those subjects at highest risk for lung cancer and treatment should be delivered promptly to such individuals. There are numerous agents that are effective inhibitors of lung carcinogenesis in animal models and these agents operate by diverse mechanisms. Chemopreventive agents with activity against carcinogens in tobacco smoke such as Nitrosamine 4 (methyl nitrosamino)-1 (3-pyridyl)-1 butanone (NNK) and Benzo(a) pyrene (B(a)P) are being tried for their effectiveness in chemoprevention of lung cancer. Development of chemopreventive agents that might be applicable to ex-smokers is also warranted. According to Hecht et al,[97] rather than a single agent that target single pathway or carcinogen, a mixture of chemopreventive agents, which target multiple carcinogens, toxicants, co-carcinogens, tumour promoters and inflammatory compounds in cigarette smoke and their downstream tumour promoting and inflammatory effects would be most successful. The WHO/International Association for the Study of Lung Cancer Classification has recognized distinct histologic lesions that can be reproducibly graded as precursors of non small cell lung cancer.[98] Chemoprevention agents are recommended as part of well designed clinical trials. In a recent review on lung cancer chemoprevention trials that are currently ongoing, majority have been reported[96] to be in phase II trials and are based on molecular pathways related to lung carcinogenesis. COX inhibitors, Iloprost, Leukotriene modifiers, selenium, green tea and broccoli sprout extracts etc are some of the agents identified as "actively recruiting" on the NIH sponsored clinical trial website (http://clinicaltrials.gov). As there is a pressing need for effective lung cancer chemoprevention, few additional trials are reported to be in the planning stages too.[96] However, smokers should be considered for chemoprevention of lung cancer, but with the strong message that no chemopreventive agent makes smoking safe. And in addition, chemoprevention should be given in the context of providing smoking cessation advice and assistance.

Smoking Cessation

Information about genetic susceptibility could be utilized in the psychological realm also. Researchers have already explored the use of genetic susceptibility information for lung cancer to motivate smoking cessation, and has been found to affect significant lifestyle or health related behavioral changes. Using two experimental factors, Mc Bride et al,[99] investigated the effects of receiving genetic susceptibility information to motivate smoking cessation. Compared to Caucasians, African Americans were more likely to increase their perceived benefits of quitting to reduce risk and their desire to quit. Findings from the reviewed studies show that information about genetic susceptibility for lung cancer increased smoker's quit attempts and affected their smoking related cognitions and emotions.[99–102] Although genetic susceptibility information increased perceived risks, motivated smoking cessation attempts, and promoted short term cessation, it was not sufficient to produce sustained abstinence especially within the minimal contact smoking cessation intervention.

Markers of genetic susceptibility to tobacco related cancers could personalize harms of smoking and motivate cessation. McBride et al[99] assessed whether a multi component intervention that include feedback about genetic susceptibility to lung cancer increased risk perceptions and rates of smoking cessation compared with a standard cessation intervention. Smokers agreed to genetic feedback as part of a multi component cessation program. Although the program increased short term cessation rates compared

with standard intervention, the study concluded that genetic feedback of susceptibility might not benefit smokers with high baseline risk perceptions. Knowledge of genetic susceptibility to lung cancer has little impact on efforts to quit smoking. Evidently, the role of genetic knowledge in lifestyle modification is quite complex and more work is needed to see how genetic tests fit into smoking cessation programmes.

Most research on the role of genetic variation on smoking cessation pharmacotherapy has been directed to two most widely accepted licensed forms of smoking cessation therapy: Nicotine replacement therapy (NRT) and the antidepressant Bupropion. It has been shown[103–105] that genetic variants in the dopaminergic system, opioid receptors, the bupropion-metabolizing enzyme CYP2B6, and the nicotine metabolizing enzyme CYP2A6, may play important roles in predicting smoking cessation responses to NRT and bupropion treatment. Since these genetic variants might also influence the response of smoking cessation pharmacotherapies, it is likely that assessment of genetic background could be a promising tool to guide selection of the most effective cessation treatment for an individual smoker. Welton et al[106] conducted a cost effectiveness analysis of genetic testing for smoking cessation based on the data available from the published pharmacogenetic studies of NRT and bupropion, and a previous cost effectivenss analysis of smoking cessation treatments. Their results indicated that the most cost effective strategy using a single gene test to aid prescription of smoking cessation pharmacotherapy is to prescribe both NRT and bupropion regardless of genotype as a first line treatment for smoking cessation. According to them, single gene tests (or very likely gene tests that test for only a handful of variants) are unlikely to be const-effective, partly because the predictive value of these tests is likely to be modest. So it seems likely that only when more pharmacogenetic data and more comparable studies become available, would it be possible to develop increasingly sophisticated cost benefit analysis of the potential value of genetic testing for tailoring of smoking cessation pharmacotherapy.

Based on recent research, it seems that genetic variants in several pathways related to smoking behavior influence success rates of smoking cessation therapies. The effects of smoking cessation therapy might thus also differ considerably in subgroups carrying certain genetic variants. Therefore a profile of genetic variants in these smoking related pathways could possibly be used to predict in advance which smoking cessation therapy is likely to be most effective for an individual smoker. This could lead to a more effective use of smoking cessation therapies, resulting in fewer side effects and increased cessation rates, and ultimately in reduced morbidity and mortality from respiratory diseases including lung cancer. However, before genetically tailored smoking cessation therapy can be implemented in clinical practice, future studies should investigate the effect of multiple susceptibility genes, as well as their mutual interactions on several smoking cessation therapies, in large scale comparable trials, in different ethnic racial groups and different sexes. Additionally, prospective trials should be setup to fully confirm the effect of the variants. Research of this kind could find solutions in which genetic susceptibility testing could be used in the health promotion and public health interventions. Thus genetic susceptibility information could be used not only to guide biological treatment, but also to affect significant lifestyle or health related behavioural changes.

Disclosure

This manuscript has been read and approved by the author. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The author reports no conflicts of interest.

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