HHS Public Access

Author manuscript

Free Radic Biol Med. Author manuscript; available in PMC 2019 October 07.

Published in final edited form as:

Free Radic Biol Med. 2015 November ; 88(Pt B): 362–372. doi:10.1016/j.freeradbiomed.2015.06.012.

FUNCTIONAL POLYMORPHISMS IN *NRF2*: IMPLICATIONS FOR HUMAN DISEASE

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Abstract

Nuclear factor (erythroid derived)-2 like 2 (NFE2L2), also known as nuclear factor erythroid 2 (NF-E2)-related factor 2 (NRF2), is a ubiquitous transcription factor essential for protecting cells and tissues from oxidative stress-induced injury. Positional cloning and studies with *Nrf2* knockout mice have identified important roles for this transcription factor in disease phenotypes for many organ systems. Studies have also characterized the means through which human *NRF2* is regulated and the mechanisms of interaction with antioxidant response elements (ARE) in promoters of effector genes. Moreover, single nucleotide polymorphisms (SNPs) in NRF2 have been identified and evaluated for effects on gene expression and function, and translational investigations have sought to determine whether loss of function SNPs associate with disease progression. In this review, we present 1) an overview of the human NRF2 gene and protein domain, 2) identification of genetic mutations in *NRF2* and associations of the mutations with multiple diseases, and 3) the role of somatic mutations in NRF2 in diseases, primarily various cancers.

Keywords

somatic; mutations; complex disease; genetic; promoter; antioxidant response element; mouse; genome-wide association

II. Introduction

NRF2 is a ubiquitous transcription factor essential in host defense [1,2]. NRF2 transcriptionally activates ARE-bearing genes in response to reactive oxygen species (ROS) produced during oxidative stress [3–6]. NRF2 homeostasis is regulated by Kelch-like erythroid-derived Cap'n'Collar Homology (ECH)-associated protein 1 (KEAP1), a cytoplasmic NRF2 suppressor [7]. In unstressed conditions, KEAP1 binds NRF2 and brings it into close proximity with Cullin 3 (CUL3) an E3 ligase which polyubiquinates NRF2 for proteasomal degradation [8]. However, electrophilic and oxidative insults are known to

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Conflict of Interests

The author declares that there are no conflicts of interest.

modify thiol residues in KEAP1, which may alter binding interactions between KEAP1, CUL3, and NRF2, and permit newly synthesized NRF2 to bypass KEAP1 inhibition and transactivate antioxidant target genes [9]. It is important to note that there exist other Keap1-independent modes of NRF2 regulation, including GSK3/betaTrCP-dependent degradation through the Neh6 domain [10]. Greater detail about the regulation of NRF2 is presented elsewhere in the series of papers in the Special Issue of *Free Radical Biology & Medicine* (reference).

Mice with targeted deletion of *Nrf2* (*Nrf2*$^{-/-}$) have been widely used to investigate the role of the transcription factor in disease models during the last decade [11–13]. Moreover, murine *Nrf2* was identified through positional cloning as a susceptibility gene in oxidative lung disorders [14,15]. Animal studies have focused on the NRF2-ARE pathway as a means to identify novel therapeutic targets for human diseases in which oxidative stress is implicated, and translational research efforts have confirmed the importance of NRF2 in oxidative disease pathogenesis and cancer progression.

The current review addresses genetic and somatic mutations in human *NRF2*. We identified and categorized genetic variations including single nucleotide polymorphisms (SNPs) and haplotypes available from the 1000 Genomes Project and the International HapMap Project databases. We have also annotated putative functional genetic polymorphisms reported to associate with disease risk. Finally, we report somatic mutations identified through targeted cohort and whole exome sequencing of cell/tissue tumor samples from neoplastic individuals.

III. NRF2 Gene and Protein Domains

Human *NRF2* is located in the cytogenetic band 2q31.2 of chromosome 2 spanning 178,095,031–178,129,859 bp (gene ID: 4780) on the reverse strand as a complementary sequence (Figure 1). NRF2 mRNA is 2,859 base pairs long (variant 1: NM_006164) and the full-length transcript encodes a protein containing 605 amino acid (aa) residues (isoform 1: NP_006155 or Q16236). Transcript variants have been reported [10]; variant 2 (NM_001145412, 2746bp) has an alternate promoter, 5'-UTR and downstream start codon. The isoform 2 protein is truncated and lacks 16 amino acids in the N-terminal region (NP_001138884 or Q16236–2, 589 aa). Isoform 3 (NP_001138885.1 or Q16236–3, 582 aa) is encoded by transcript variant 3 (NM_001145413.2; 2,725 bp), and 16 amino acids in the N-terminal region as well as an internal segment (7 amino acid) in exon 4 are missing due to alternate splicing, compared to isoform 1.

The NRF2 protein consists of six NRF2-ECH homology (Neh) domains (Figure 1). The Neh2 and Neh1 domains are the most highly conserved and most extensively studied [9,16,17]. The Neh2 domain mediates KEAP1 repression through the DLG/ETGE motif (17–32 aa, 77–82 aa) which binds the DC motif of KEAP1 for redox-sensitive proteasomal degradation. Neh4 and Neh5 act as translocation and transactivation domains through interaction with CREB (cAMP Response Element Binding Protein). The Neh6 domain may contain a degron motif involved in NRF2 turnover and KEAP1-independent degradation. Neh1 possesses basic leucine zipper motifs (503–518 aa, 525–539 aa) for dimerization and

DNA binding. The Neh3 domain may play a role in stability and act as a transactivation domain through interaction with the transcriptional machinery.

IV. Genetic mutations of *NRF2* and association with disease risk

Polymorphisms and haplotype alleles

Genome-wide association studies (GWAS) have examined SNPs across the genome to identify 'risk' genotypes significantly more prevalent in an affected group for disease association. Supporting GWAS, the 1000 Genomes Project has sequenced more than 2000 genomes (~2500 to date) of individuals with diverse ethnicity. The HapMap Project has also mapped combinations of alleles at specific loci (haplotypes) to generate DNA sequence variation patterns that contribute to disease risk. In conjunction with the 1000 Genomes project, this provides efficient mapping of multiple loci for complex traits, and offers a key resource to locate disease susceptibility genes.

Using these publically available tools, we profiled more than 583 sequence mutations of the *NRF2* locus, including five in the 5'UTR/proximal promoter, fifty-nine exonic SNPs [26 non-synonymous coding (Cns) mutations] and a triplet repeat variation $GGC_{4–5}$ [18,19]. We then filtered for functional polymorphisms associated with disease risk (Table 1: 5'UTR, exonic, intronic, and 3' distal SNP alleles are presented as chromosome contig alleles on the forward strand, while location on *NRF2* is presented as reverse contig alleles; subsequent references in the text will use the forward sequence exclusively). Genotypes that conferred risk were largely non-exonic and located in 5' flanking regions and introns, suggesting they affect *NRF2* expression. Association of *NRF2* sequence variations with specific disease subtypes reported in epidemiological studies are summarized in Table 2.

Linkage disequilibrium (LD) is a measurement of genetic loci that exist in proximal space, and regions with high LD indicate chromosomal segments likely to be inherited together during DNA recombination. This can be attributed to genetic linkage, that is, regions close in space on the same chromosome, or through functional interactions between alleles that confer selective advantage. *NRF2* linkage disequilibrium data from Haploview (http://www.broadinstitute.org) and previous work [20] are depicted in Figure 2A (bottom and top panels, respectively). Chromosome 2 locations are relative to NC_000002.12 and assembly GRCh38.p2, and SNP positions relative to NM_006164 (transcript variant 1). Haplotype blocks for *NRF2* tagging SNPs from 10 ethnically diverse individuals (Asian, African, European and African Americans) are displayed in Figure 2B (modified from http://snpinfo.niehs.nih.gov/snpinfo/snptag.htm).

Association with respiratory diseases.

Intronic and promoter *NRF2* SNPs have been investigated for association with respiratory diseases including acute lung injury (ALI), cigarette smoke-induced chronic obstructive pulmonary disease (COPD), and asthma. The rs6721961 G to T substitution at −178 of the proximal promoter associated with increased risk of ALI following major trauma in European and African-Americans (odds ratio, OR 6.44; 95% confidence interval, CI 1.34–30.8) [21]. Promoter activity was assessed *in vitro* using luciferase reporter assays, and

mutation to the T allele significantly reduced transcriptional activity compared to wild-type G allele constructs. As the SNP occurs within an ARE-like motif, this suggests an auto-regulatory role for variation at this locus [21]. The variant rs6721961 T allele was also nominally associated with ALI-related 28-day mortality following systemic inflammatory response syndrome [22]. In contrast, Japanese individuals with the haplotype (rs6721961 T/rs2364722 A/rs1962142 A/rs6726395 A/rs2001350 T) were protected from FEV_1 decline (forced expiratory volume in one second) in relation to cigarette smoking status ($p = 0.004$) suggesting modulation through gene by environment interaction [23,24].

Using an additive model, a significant interaction was identified between rs6726395 (G/G + G/A) genotyped individuals, smoking status and reduced mean FEV_1 in the same Japanese cohort ($p_{int} = 0.010$) [23,24]. Similarly, the intronic haplotype rs2364723 C/rs6726395 A was associated with significantly reduced FEV_1 in Japanese smokers with lung cancer [25], supporting work by Siedlinski *et al.* [26] who found lower FEV_1 levels in the Dutch Vlagtwedde-Vlaardingen cohort of 1152 individuals (p = 0.04; CI −87.3 to −1.7). In addition, individuals with the intronic haplotype (rs2364723 C/rs13001694 G/rs1806649 C/rs4243387 T/rs6726395 G) had high FEV_1 levels compared to smokers [26]. In a follow-up study from the Netherlands (n = 1390 cases), rs1806649 T allele carriers had significantly increased COPD survival [hazard ratio (HR) 0.5; CI 0.3–0.7] while rs13001694 G and rs2364723 C allele carriers had a tendency toward increased all-cause and cardiovascular mortality, respectively [27].

In a Hungarian population of childhood asthma, a trend toward protection from infection-induced asthma (OR 0.290; p = 0.015) was found in children with the rs6721961 G allele, and significant interaction of the *NRF2* promoter SNP with moderate nitrogen dioxide exposure was found (OR 0.43; CI 0.23–0.83) [28]. Canova *et al.* [29] reported an association with increased hospital admission rate during high-level particulate matter (PM_{10}) exposure that significantly correlated with the intronic rs1806649 C allele in a Caucasian cohort (OR 1.35; CI 1.04–1.76). Asthma and COPD admission rates were related to increased environmental PM_{10} concentrations. Interaction between prenatal stress and *NRF2* SNPs was investigated in the Avalon Longitudinal Study (United Kingdom). While maternal smoking during pregnancy did not associate with lung function change or with asthma incidence in school-aged children, as this relation was not modified by *NRF2* status [30], early gestational acetaminophen exposure significantly influenced the risk of asthma and wheeze at age 7 in more than 5,000 children [31]. When maternal copies of the rs6706649 T allele were present, association with asthma (OR 1.73; CI 1.22–2.45) and wheeze (OR 1.53; CI 1.06–2.20) were significantly increased [31].

Interestingly, the homozygous rs6721961 C/C allele at the promoter −178 in cohorts of very low birth-weight (VLBW) infants was significantly ($p < 0.01$) associated with decreased severe bronchopulmonary dysplasia (BPD) [32].

Association with cardiovascular diseases.

NRF2 promoter SNPs have been examined for association with cardiovascular disease (CVD), a multi-factorial disorder with both genetic and environmental risk factors. Diabetics with the rs35652124 T promoter allele had an increased risk of CVD in a Japanese cohort

(OR 2.834; p = 0.006) [33]. Similarly, rs6721961 T allele carriers in the ESTHER study that were given oral estrogen had increased risk of venous thromboembolism (OR 17.9; CI 3.7–85.7) [34]. SNPs in *NRF2* intron 1 were associated with CVD in the Vlagtwedde-Vlaardingen cohort. Using an additive model, Figarska *et al.* [27] showed a protective effect of the rs2364723 C allele mediated by reduced triglyceride levels (HR 0.49; CI 0.33–0.74). In contrast, the distal intronic rs1806649 SNP was associated with increased risk of atypical embryonic heart development. Chinese infants with the heterozygote rs1806649 C/T genotype had increased risk of congenital heart disease (OR 1.84; CI 1.03–3.29) suggesting *NRF2* hotspots may regulate heart development and modulate cardiac injury through *NRF2* transactivation [35].

Vasodilation, or widening of the blood vessels, occurs via smooth muscle cell relaxation of the arteries, veins and smaller arterioles for efficient oxygen delivery to organs and localized tissues during high levels of metabolic activity. During atherogenesis, recurrent diminished vasodilatory response due to endothelial dysfunction can clinically manifest as myocardial infarction and stroke [36]. Oxidative stress can mediate endothelial damage and injury, aiding progression of atherosclerosis. Marczak *et al.* [36] found an association of the *NRF2* rs35652124 C allele and reduced forearm blood flow (FBF; $p < 0.001$) with increased forearm vasodilator response (FVR; $p = 0.006$) in healthy African Americans in response to bradykinin or sodium nitroprusside. Similarly, the rs6721961 T allele increased FVR ($p = 0.035$) in healthy Caucasians recruited for the same study. Blood pressure was also modulated by *NRF2* interaction. Increased systolic ($p \leq 0.001$) and diastolic blood flow ($p \leq 0.039$) was shown in Japanese hemodialysis patients with the *NRF2* haplotype rs35652124 C/T/rs6706649 C/T [33].

Association with gastrointestinal disorders.

Recurrent *Helicobacter pylori* (*H. pylori*)-induced gastritis often progresses into gastric atrophy and cancer. Studies in Japanese populations suggested an association of *NRF2* variations with gastrointestinal tumorigenesis. The promoter rs35652124 C/rs6706649 C haplotype was significantly associated with increased p14 methylation following *H. pylori* infection (OR, 2.90; 95% CI 1.14–7.36) [37]. Subsequent studies identified rs35652124 T/rs6706649 C allele carriers with increased inflammation scores ($p < 0.041$) and a tendency toward increased severity of gastric mucosal atrophy [38]. The rs35652124 C/rs6706649 C haplotype was protective for gastrointestinal disease (OR 0.45; CI 0.22–0.93) while individuals heterozygous at each locus (rs35652124 C/T/rs6706649 C/T) were susceptible to ulcerative colitis in a Japanese population (OR 2.57; CI 1.01–6.60) [39].

Association with neurodegenerative diseases.

The etiology of Parkinson's disease correlates with increased oxidative stress, through production of ROS via dopamine metabolism and low levels of antioxidants in the substantia nigra of the brain. von Otter *et al.* [40] evaluated *NRF2* promoter haplotypes (rs35652124 T/rs6706649 C/rs6721961 G) and intronic haplotypes (rs7557529 C/rs2886161 T/rs1806649 T/rs2001350 T/rs10183914 C) in Swedish and Polish Parkinson's patients enrolled in the PD-Goth and PD-Link studies. The *NRF2* promoter haplotype was protective for Parkinson's disease (OR 0.6; CI 0.4–0.9) while the intronic haplotype conferred increased

susceptibility in Swedish individuals (OR 3.7; CI 1.3–10.6) [40]. *NRF2* haplotype alleles were associated with 2 years earlier age at onset (AAO) of Alzheimer's disease, 4 years earlier AAO of posterior sub-capsular cataract surgery, and 4 years later AAO of cortical cataract surgery [41].

Meta-analysis of the extended cohort (case size 1038, including Italian, Maltese and German patients) identified individual *NRF2* risk alleles. While intronic rs7557529 T and rs2886161 C, and promoter rs35652124 C alleles increased Parkinson's disease risk with earlier age at onset of disease individually [(AAO −1.0yrs; CI −1.94 to −0.03), (AAO −1.2yrs; CI −2.27 to −0.18) and (AAO −1.2yrs; CI −2.12 to −0.02) respectively], the intronic rs1806649 T allele was protective for Parkinson's disease and increased AAO to +1.2yrs (CI 0.12 to 2.28) [20]. *NRF2* is also a potential susceptibility gene in degenerative motor neuron diseases including amyotrophic lateral sclerosis (ALS, also known as Lou Gehrig's disease) in which oxidative stress is involved. Bergstrom *et al.* [42] demonstrated that the compound *NRF2* haplotype (rs7557529 C/rs35652124 T/rs6706649 C/rs671961 G/rs2886161 T/rs1806649 C/rs2001350 T) was associated with decreased risk of sporadic ALS in a Swedish cohort (AAO +4.0yrs; CI 1.1 to 7.0).

Association with cancer

In female Japanese non-smokers, the rs6721961 T allele in the proximal *NRF2* promoter was significantly associated with lung adenocarcinoma susceptibility ($p = 0.014$) [43]. This SNP was four-fold more frequent in females than males, and regarded as a genetic biomarker to assess overall survival ($p = 0.021$ in smokers, + 1000 days). Cholangiocarcinoma (CCA) is a malignancy of the biliary ducts with poor survival rates. A Thai cohort (n = 198) was studied for the association of *NRF2* mutations with CCA risk and their impact on cancer survival. The intronic rs6726395 G allele was protective and associated with longer survival (HR 0.54; CI 0.31–0.94) suggesting a potential prognostic biomarker for CCA patients [44].

Estrogen metabolites (e.g., catechols) generate ROS and cause oxidative stress suggesting association of *NRF2* variants and downstream effectors in postmenopausal mammary cancer. In a Finish population, rs6721961 T allele carriers (OR 4.656; CI 1.35–16.06) and individuals with the rs2706110 A allele were at increased risk of breast cancer (OR 2.079; CI 1.175–3.679), while those bearing the 5' flanking rs2886162 A allele had significantly reduced survival (HR 1.687; CI 1.1047–2.748) [45]. The rs6721961 A allele carriers together with intronic rs1962142 A allele carriers had reduced NRF2 expression in breast tissue [45]. The intronic rs1806649 SNP did not associate with breast cancer risk in postmenopausal women [46]. However, when this SNP and other risk alleles of ARE-responsive genes including NAD(P)H:quinone oxidoreductase (*NQO1*) and heme oxygenase 1 (*HO1*) were combined, the risk was significantly increased (P trend = 0.04) with those having three or more high-risk alleles at 56% greater risk of breast cancer compared with the referent group (OR, 1.56; 95% CI, 0.97–2.51) [46].

Associations with other disorders.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease more frequently found in females than in males. It affects organs such as skin, joints, kidneys, and brain, and recurrent inflammation can lead to nephritis of the glomeruli and tubules of the kidneys. GWAS in humans identified a suggestive quantitative trait locus near *NRF2* [47]. In a Mexican Mestizo population of 362 females with childhood-onset SLE (212 with nephritis) the rs35652124 C/T genotype significantly associated with nephritis (OR 1.81; CI 1.04–3.12) [48]. The same SNPs were not closely associated with SLE risk in a Japanese cohort [18]. Vitiligo is a skin condition in which there is a loss of pigment from areas of skin, resulting in irregular white patches. It is thought to be an autoimmune disease caused by loss of melanocytes that produce brown pigment. The rs6721961 T allele increased risk of vitiligo in a Chinese Han population (OR 2.902; CI 1.624–5.188) [49]. Age-related macular degeneration (AMD) is one of the most common causes of vision loss in the elderly. Decreased risk of AMD wet form (OR 0.44; CI 0.23–0.85) was found in rs6726395 G allele carriers, and this SNP decreased disease susceptibility (OR 0.35) in conjunction with the *NOS3* rs1799983 polymorphism [50].

V. Somatic *NRF2* mutations

Recent research has provided significant insight into mutagenesis and cancer development in various organs such as the lung. Somatic or acquired mutations change the genetic structure of diploid cells but are not heritable. Together with epigenetic changes (epimutations), somatic mutations predispose individuals to cancer through changes in the activity of affected genes. Six patterns of somatic mutations, C>A/G>T, C>G/G>C, C>T/G>A, T>A/A>T, T>C/A>G, and T>G/A>C, have been established in the cancer genome [51].

Somatic *NRF2* mutations were initially identified in Asian lung cancer cohorts. However, recent advances in sequencing technologies have enabled high-resolution examination of cancer genomes. Whole exome sequencing has revealed that the six patterns of somatic mutations are ubiquitous in cancers from diverse ethnic groups [52–54]. Interestingly, somatic *NRF2* mutations are clustered exclusively in the DLG/ETGE motifs of the Neh2 domain (hinge and latch region, Figure 1) that is essential for KEAP1 binding and repression [9]. We have summarized somatic *NRF2* mutations in various human cancer cells and tissues that were revealed by whole-genome or -exome sequencing or by targeted DLG/ETGE motif sequencing (Table 3). A somatic mutation database (COSMIC, http://cancer.sanger.ac.uk/cosmic/search?q=nfe2l2) contains an extended list of *NRF2* mutations (Supplemental Table 1). The list includes mutations in the DLG/ETGE motif of the Neh2 domain as well as mutations in other domains (e.g., p.G345S in Neh6 domain), however many of these non Neh2 domain mutations have not been investigated to characterize their functions or association with adverse disease outcome. Somatic NRF2 mutations lead to gain of activity, which causes excess accumulation and aberrant transactivation of NRF2 due to suppression of its ubiquitination through KEAP1 binding. These mutations are hypothesized to aid survival and chemotherapy resistance of cancer cells.

NRF2 somatic mutations often occur coincidentally with *KEAP1* and *CUL3* mutations in lung cancer [55,56] and in other cancers such as esophageal squamous cell carcinoma

(ESCC [53]) and type 2 papillary renal cell carcinoma [57]. A large-scale non-small cell lung cancer (NSCLC) cohort was sequenced for The Cancer Genome Atlas [58], and a very high mutation rate (34%, 60/178 cases) was identified in DLG/ETGE motifs of *NRF2* (19%), and in motifs of *KEAP1* (12%) and *CUL3* (7%), consistent with the significance of oxidative stress and the NRF2 pathway in oncogenesis. Somatic *KEAP1* mutations cause loss of KEAP1 function which enhances NRF2 accumulation [59,60]. In NSCLC and gastrointestinal, breast, and prostate cancers, multiple germline *KEAP1* Cns mutations cause dysfunction of the translated protein and increased accumulation of NRF2 and risk of neoplasia and chemo-resistance [9,56,61,62].

NRF2 mutations have been significantly associated with NSCLC cases (adenocarcinoma and squamous cell carcinoma), particularly in Japanese (10.7%, [55]), Chinese (23%, [63]), and Koreans (8% [64]), as well as lung cancer cell lines. Smoking history correlated with mutation rate in all studies [55,63–65]. Lung cancer patients with somatic *NRF2* mutations had significantly worse prognosis (rate of mortality and mean survival time) relative to patients with wild-type *NRF2* sequence [65].

In addition to lung cancer, laryngeal squamous carcinoma (13% in [64]), ESCC (22% in [63], 11.4% in [64], 10% in [53]; 4.5% in [66]), head and neck cancers (25% in [55]), skin (1/17 case in [64]), and oral cancer cell lines contained somatic changes in *NRF2*. In hepatocellular carcinoma, *NRF2* mutations were recurrent and present in 6.4% of cases [67]. Somatic *NRF2* mutations were found in 13% of childhood hepatoblastoma cases using whole exome sequencing, and targeted genotyping of primary tumors and hepatoblastoma cell lines confirmed mutations in 9.8% of cases [54]. Exome sequencing in primary squamous cell cervical carcinoma patients identified four somatic *NRF2* mutations (4%), three with recurrent frequency [52]. A pilot study from a Russian primary liver cancer cohort revealed two additional somatic *NRF2* mutations in one case (2.9%; [68]).

The most variable sites in NRF2 include negatively charged amino acid residues (Asp29, Asp77, and Glu79) and the hydrophobic Gly31 residue. Importantly, Ser33 in the Neh2 domain can be modified through genetic or somatic processes [69]. Functional analyses of the Cns in the ETGE motif of *NRF2* demonstrated that mutants have impaired recognition of KEAP1 [55]. RNAi-mediated depletion of *NRF2* in lung cancer cells enhanced ROS production and susceptibility to cell death by ionizing radiation [70], and *NRF2* somatic mutations were significantly correlated with increased copy number of the NRF2 gene in NSCLC cases [71]. Furthermore, ‘activating’ somatic *NRF2* mutations increased expression of downstream effectors including RagD, a known mediator of squamous cell proliferation in the lung [72].

In summary, ‘gain-of-function’ somatic mutations in *NRF2* alone or coincidentally with ‘loss-of-function’ mutations in *KEAP1-CUL3* aberrantly increase NRF2 levels, and are suggested to be predictive markers for malignant progression and poor responsiveness to chemotherapy. Although NRF2-mediated cellular defense is essential during cancer initiation, constitutive NRF2-ARE activity in cancer progression may create a favorable intracellular environment for tumor cell growth and survival [73,74]. In addition to targeted sequencing, continued large-scale whole genome and exome sequencing will provide

comprehensive understanding of genomic alterations in oncogenesis. In conjunction with related functional neoplastic networks this can enhance discovery of targetable somatic mutations in cancer etiology.

VI. Conclusions

Studies using mice with targeted deletion of *Nrf2* have yielded valuable insight to the role of this transcription factor in health and disease in multiple organ systems, and potential understanding of factors that contribute to human diseases. Subsequent investigations that have characterized the genetic and molecular function of human *NRF2*, including associations of *NRF2* SNPs with disease phenotypes, have also provided novel targets for disease prevention. A limitation of the association studies is that many are under-powered and need to be replicated to better investigate the role that *NRF2* has in disease progression. Future studies should also be designed to query interaction between NRF2 and other transcription factors (e.g. NF-κB), as well as NRF2 effector molecules (e.g. NQO1), in order to better understand the mechanisms through which these genes modulates disease susceptibility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The research related to the manuscript was supported by the Intramural Research Program of the National Institutes of Health, National Institute of Environmental Health Sciences (NIEHS). Drs. Michael Fessler and Donald Cook at the NIEHS provided excellent critical review of the manuscript.

Abbreviations:

AAO	age at onset
ALI	acute lung injury
AMD	age-related macular degeneration
ARE	antioxidant response element
BPD	bronchopulmonary dysplasia
β-TrCP	β-transducin repeats-containing proteins
CCA	cholangiocarcinoma
Cns	non-synonymous coding
COPD	chronic obstructive pulmonary disease
COSMIC	catalogue of somatic mutations in cancer
CREB	cAMP response element binding protein

CUL3	cullin 3
CVD	cardiovascular disease
ESCC	esophageal squamous cell carcinoma
FBF	forearm blood flow
FEV_1	forced expiratory volume in one second
FVR	forearm vasodilator response
GSK3	glycogen synthase kinase 3
GWAS	genome-wide association studies
HO1	heme oxygenase 1
KEAP1	kelch-like erythroid-derived Cap'n'Collar homology (ECH)-associated protein 1
LD	linkage disequilibrium
Neh	NRF2-ECH homology
NFE2L2	nuclear factor (erythroid derived)-2 like 2
NQO1	NAD(P)H:quinone oxidoreductase
NRF2	nuclear factor erythroid 2 (NF-E2)-related factor 2
NSCLC	non-small cell lung cancer
ROS	reactive oxygen species
SLE	systemic lupus erythematosus
SNP	single nucleotide polymorphism
UTR	untranslated region
VLBW	very low birth-weight

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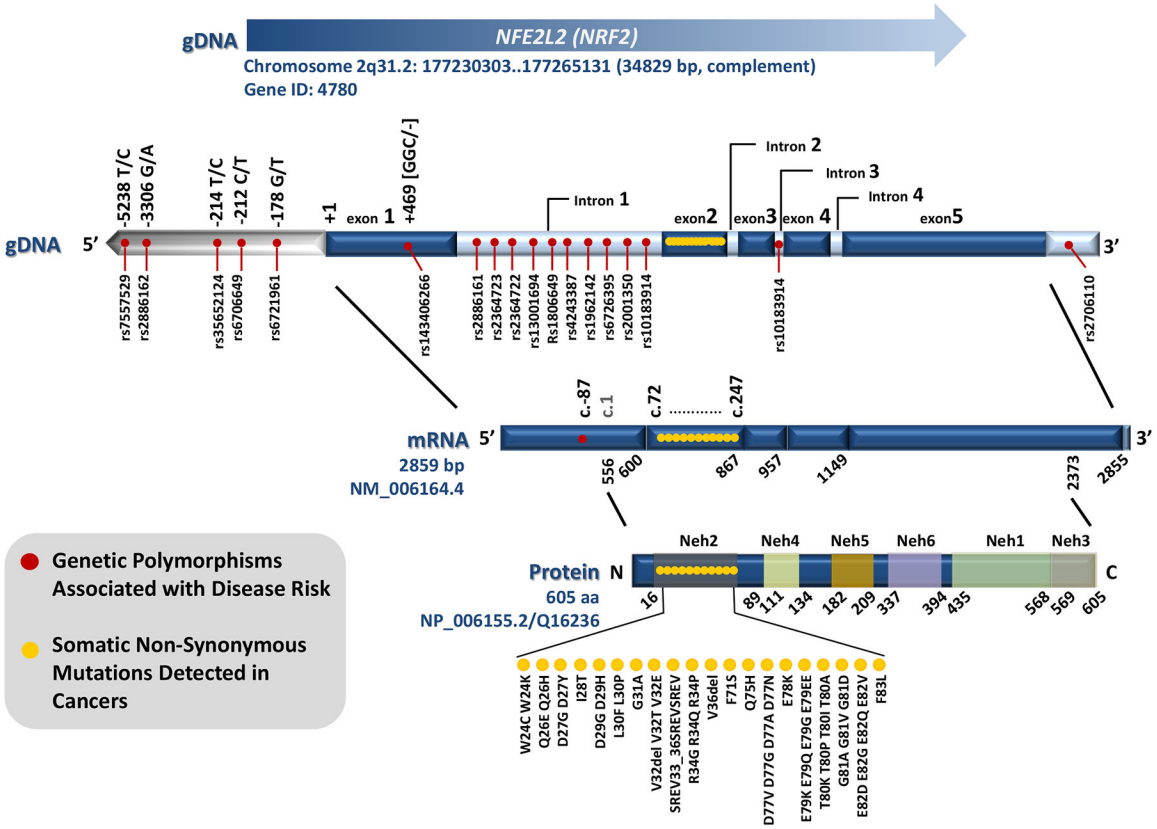

Figure 1. Human NRF2 gene, mRNA, and protein.

Human *NRF2* located on human chromosome 2 harbors 5 exons, and transcript (variant 1, 2856 bp) encodes a protein containing 605 amino acid residues. NRF2 protein is consisted of 6 highly conserved Neh domains. At-risk genetic variations in the promoter, exon 1, and introns of *NRF2* are depicted by red dots. Somatic, non-synonymous mutations clustered in Neh2 domain associated with various cancers are marked as yellow dots.

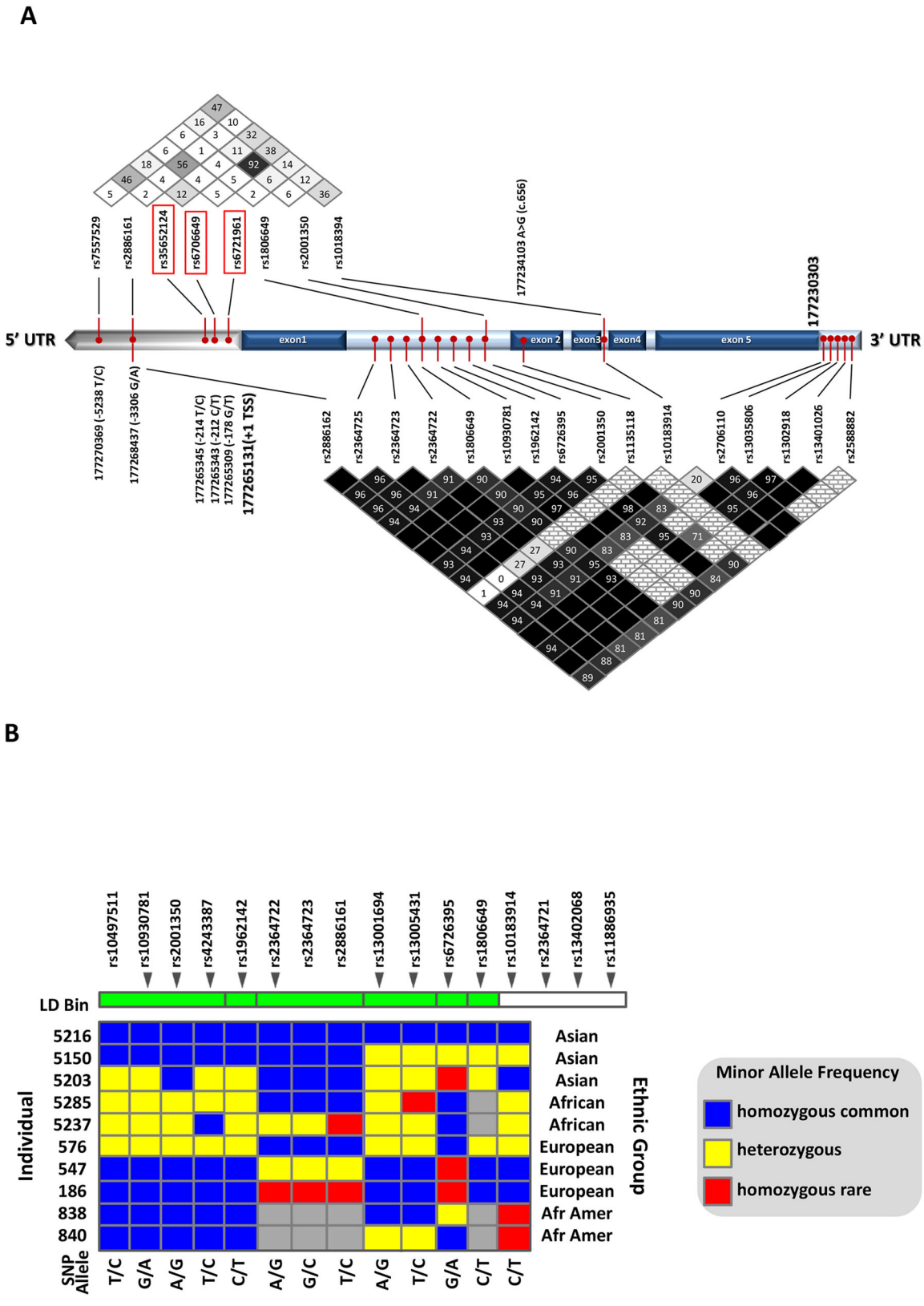

Figure 2. Haploview linkage disequilibrium (LD) plot of *NRF2* SNPs.

(A) Linkage disequilibrium (LD) data for *NRF2* SNPs acquired from Haploview [http://www.broadinstitute.org] and other publications [24]. Intensity of shading and numerical values indicate level (or strength) of linkage between two comparison loci and patterned squares indicate loci with insufficient linkage data, while low LD values can indicate regions of homologous recombination or crossover during mitosis. Three proximal *NRF2* promoter SNPs (rs6721961, rs6706649, and rs35652124) in the red rectangles are involved in a variety of diseases detailed in this review (**Details in** Table 3), and ordered relative to the transcription start site (TSS). UTR = untranslated region. Refer to NC_000002.12 for chromosome 2 location. Referred to NM_006164 (transcript variant 1) for promoter and exon SNP location. **(B)** *Nrf2* SNP haplotypes blocks modified from http://snpinfo.niehs.nih.gov/snpinfo/snptag.htm. Ten representative individuals from ethnic groups (Asian, African, European and African Americans) are shown for color-coded haplogroup blocks. Blue is the major allele or homozygous common genotype, red is the minor allele or homozygous rare genotype, and yellow is heterozygous at that locus. Gray signifies missing data at this locus. Arrows denote individual tagging SNPs as shown at top in linkage disequilibrium (LD) bins.

Table 1.

Genetic polymorphisms in *NRF2* associated with disease risk.

dbSNP ID (Prior ID)	Chromosome 2 Locus* (GRCh38.106)	Minor allele frequency/count (1) 1000 Genomes (2) 1000 Genomes + HapMap+	Loation on *NFE2L2* (Gene ID: 4780)
rs7557529	177270369 T/C	(1) C = 0.3952/1978	5' flanking (−5238 G>A)
rs2886162	177268437 G/A	(2) A = 0.3960/1982	5' flanking (−3306 T>C)
rs35652124 (rs57695243)	177265345 T/C	(1) C = 0.3756/1880	5' promoter (−214 A>G) [formerly −653 or −686]
rs6706649	177265343 C/T	(1) T = 0.0633/316	5' promoter (−212 G>A) [formerly −651 or −684]
rs6721961 (rs117801448)	177265309 G/T	(2) T = 0.1452/727	5' promoter (−178 A>C) [formerly −617 or −650]
rs143406266	177264663 [-/GGC]	Not reported	Exon 1 (+467/+469)
rs2886161	177263111 T/C	(2) C = 0.3764/1885	Intron 1 (+2022)
rs2364723	177261818 G/C	(2) C = 0.3730/1867	Intron 1 (+3315)
rs2364722	177260059 A/G	(2) G = 0.3740/1873	Intron 1 (+5074)
rs13001694	177254262 A/G	(1) G = 0.2448/1225	Intron 1 (+10871)
rs1806649 (rs58745895)	177253424 C/T	(1) T = 0.1052/527	Intron 1 (+11709)
rs4243387 (rs60038464)	177253037 T/C	(2) C = 0.2933/1469	Intron 1 (+12096)
rs1962142 (rs58448508)	177248756 G/A	(2) A = 0.1120/561	Intron 1 (+16377)
rs6726395 (rs57309289)	177238501 G/A	(2) A = 0.4291/2148	Intron 1 (+26632)
rs2001350 (rs17515179) (rs60883775)	178100425 T/C	(2) C = 0.1282/642	Intron 1 (+29436)
rs10183914 (rs58731187) (rs61374844)	177232938 C/T	(2) T = 0.2318/1160	Intron 3 (+32195)
rs2706110	177227434 C/T	(2) T = 0.3317/1660	3'flanking

*First allele is major (wild type), second allele is minor (variant).

+Variations from samples shared by both projects.

Table 2.

Functional *NRF2* polymorphisms and haplotypes.

	Disorder	Reference	SNP/Haplotype[+]	Population (cases)	OR, HR, AAO, CI
Respiratory	**COPD** **COPD/Asthma (PM 10)**	2014 [27] 2012 [29]	rs1806649 T rs1806649 C	Caucasian/Dutch (1390) British (209)	↓Cardiovascular mortality HR 0.5 (CI 0.3 to 0.7) OR 1.35 (CI 1.04 to 1.76)
	Annual FEV_1 decline **FEV_1 decline in lung cancer**	2011 [23,24] 2009 [75] 2013 [25]	[+]rs6721961 T/rs2364722 A/rs1962142 A/rs6726395 A/rs2001350 T rs6726395 (GG + GA) rs1806649 T [+]rs2364723 C/rs6726395 A	Japanese smokers (896) Caucasian/Dutch (1152) Japanese smokers (209)	↓ FEV_1 decline (p = 0.004) ↑ FEV_1 decline (p = 0.010) ↓ FEV_1 −44.5 mls [p = 0.04 (CI −87.3 to −1.7)] ↓ mean FEV_1 (p = 0.05)
	Acute lung injury	2007 [21] 2012 [22]	rs6721961 G/T rs6721961 T	Caucasian/Afr. American (30) Caucasian (224)	OR 6.44 (CI 1.34 to 30.8) p = 0.021 28 day mortality OR 9.73 (CI 1.27 to 74.8) p = 0.030
	Asthma	2010 [31] 2012 [28]	rs6706649 (T/C + T/T) rs6721961 (T/G + T/T)	British (1137) Hungarian/Gypsy (307)	+ prenatal acetaminophen OR 1.73 (CI 1.22 to 2.45) OR 0.437 (CI 0.28 to 0.80)
	BPD	2015 [32]	rs6721961		
Cardiovascular/ Circulatory	**Cardiovascular disease** **Thromboembolism** **Coronary heart disease**	2014 [33] 2014 [27] 2011 [34] 2013 [35]	rs35652124 T rs2364723 (G/C + C/C) rs6721961 T rs1806649 C/T	Japanese diabetics (60) Caucasian/Dutch (1390) Caucasian females (161) Chinese infants (160)	OR 2.834, p = 0.006 HR 0.49 (CI 0.33 to 0.74) + oral estrogen OR 17.9 (CI 3.70 to 85.70) OR 1.84 (CI 1.03 to 3.29) p = 0.038
	Vasodilation **Blood pressure**	2012 [36] 2014 [33]	rs35652124 C rs6721961 T rs6721961 T and rs3562124 T	Afr. American (64) Caucasian (184) Japanese (60)	↓ FBF, p < 0.001; Higher FVR, p = 0.006 ↑ FVR, p = 0.035 ↑ Systolic (p ≤ 0.001) and ↑ diastolic (p ≤ 0.039)
GI	***H pylori* infection** **Ulcerative colitis**	2007 [76] 2008 [38]	[+]rs35652124 C/rs6721961 G [+]rs35652124 C/rs6706649 C [+]rs35652124 C/T/rs6706649 C/T	Japanese (159) Japanese (89)	↑ Inflammation score, p ≤0.041 OR 2.57 (CI 1.01 to 6.60) p = 0.043 OR 0.45 (CI 0.22 to 0.93) p = 0.029
Neurodegenerative	**Parkinson's disease**	2010 [40] 2014 [20]	[+]rs35652124 T/rs6706649 C/ rs6721961 G [+]rs7557529 C/rs2886161 T/rs1806649 T/rs2001350 T/rs10183914 C rs7557529 T rs35652124 C rs2886161 C rs1806649 T	Caucasian/Polish (192) Caucasian/Swedish (165) Polish/Italian/Maltese/ German (1038) meta-analysis	OR 0.6 (CI 0.4 to 0.9) OR 3.7 (CI 1.3 to 10.6) AAO −1.0 yrs (CI −1.94 to −0.03) p = 0.042 AAO −1.1 yrs (CI −2.12 to −0.02) p = 0.045 AAO −1.2 yrs (CI −2.27 to −0.18) p = 0.021 AAO +1.2 yrs (CI 0.12 to +2.28) p = 0.029
	Amyotrophic lateral sclerosis (Lou-Gehrig's disease)	2014 [42]	[+]rs7557529 C/rs35652124 T/ rs6706649 C/rs671961 G/rs2886161 T/ rs1806649 C/rs2001350 T	Caucasian/Swedish (522)	AAO +4.0 yrs (CI 1.1 to 7.0) p = 0.008

	Disorder	Reference	SNP/Haplotype[+]	Population (cases)	OR, HR, AAO, CI
Cancer	**Adenocarcinoma** **Cholangiocarcinoma** **Breast cancer**	2013 [43] 2014 [44] 2012 [77]	rs6721961 T rs6726395 G rs2886162 A rs6721961 T rs2706110 A	Japanese (387) Thailand (198) Finnish (452)	Survival +1000 days, p = 0.021 (smokers) Adenocarcinoma p = 0.014 (non-smoking females) HR 0.54 (CI 0.31 to 0.94) ↓ Survival HR 1.687 (CI 1.047 to 2.748) OR 4.656 (CI 1.350 to 16.063) OR 2.079 (CI 1.175 to 3.679) p = 0.011
Other	**Metabolism and mortality** **SLE** **Vitiligo** **AMD**	2014 [27] 2010 [48] 2008 [78] 2013 [50]	rs13001694 (A/G + G/G) rs35652124 C/T rs6721961 T rs6726395 G	Caucasian/Dutch (1390) Mexican Mestizo females (362) Chinese (300) Caucasian/Polish (281)	All-cause mortality HR 0.77 (CI 0.59 to 1.0) Nephritis OR 1.81 (CI 1.04 to 3.12) p = 0.032 OR 2.902 (CI 1.624 to 5.188) OR 0.44 (CI 0.23 to 0.85) p = 0.039

SNP alleles correspond to GRCh38.106 annotation and refer to the forward strand allele (*NFE2L2* lies on reverse strand).

[+]Denotes haplotype. COPD = chronic obstructive pulmonary disease, GI = gastro-intestinal, PM = particulate matter, BPD = bronchopulmonary dysplasia, SLE = systemic lupus erythematosus, AMD = Age-related macular degeneration, OR = odds ratio, HR = hazard ratio, CI = confidence interval, FEV_1 = forced expiratory volume in 1 second, FBF = forearm blood flow, FVR = forearm vasodilator response, AAO = age at onset.

Table 3.

Somatic *NRF2* mutations in human cancers.

Domain	Amino acid residue			DNA mutation	Cancer type	References
	locus	Wild Type	Mutant			
DLG Motif	**24**	W (Trp)	C (Cys)	c.72 G>C/G>T	NSCLC, neck, ESCC	[52,55,79]
			K (Lys)	c.72 T>C	ESCC	[79]
	26	Q (Gln)	E (Glu)	c.76 C>G	NSCLC, ESCC	[55,79]
			H (His)	c.78 A>C	HCC	[80]
	27	D (Asp)	G (Gly)	c.80 G>A	NSCLC	[63]
			Y (Tyr)	c.79 G>T	ESCC	[64]
	28	I (Ile)	T (Thr)	c.83 C>T	NSCLC	[55]
	29	D (Asp)	G (Gly)	c.86 A>G	Head and neck, ESCC	[55,79]
			H (His)	c.85 G>C	NSCLC, larynx	[63–65]
	30	L (Leu)	F (Phe)	c.88 C>T	NSCLC, ESCC	[55,79]
			P (Pro)	c.89 T>C	Hepatoblastoma	[54]
	31	G (Gly)	A (Ala)	c.92 G>C	NSCLC, ESCC, skin	[55,63–65,79] [81]
	32	V (Val)	Del	c.93_95 del AGT	ESCC	[64]
			T (Thr)	c.95 T>G	NSCLC	[55]
			E (Glu)	c.95 T>A	Primary liver cancer	[68]
	33–36	S-R-E-V	S-R-E-V-S-R-E-V*	c.97_108 dup AGTCGAGCCGTA	ESCC	[64]
	36	V (Val)	Del	c.105_107 del GTA	PRCC2	[82]
	34	R (Arg)	G (Gly)	c.100 A>G	NSCLC, hepatoblastoma	[54,65]
			Q (Gln)	c.101 G>A	NCSCL	[52,55,63,64]
			P (Pro)	c.101 G>C	NSCLC, hepatoblastoma	[52,54,71]
ETGF Motif	**71**	F (Phe)	S (Ser)	c.212 T>C	Primary liver cancer	[68]
	75	Q (Gln)	H (His)	c.225 A>C	Head and neck, ESCC	[55,79]
	77	D (Asp)	V (Val)	c.230 A>T	NSCLC, ESCC	[55,63,79]
			G (Gly)	c.230 A>G	ESCC	[79]
			A (Ala)	c.230 A>C	NSCLC	[64]
			N (Asn)	c.229 G>A	Larynx	[64]
	78	E (Glu)	K (Lys)	c.232 G>A	NSCLC, ESCC	[55,79]
	79	E (Glu)	K (Lys)	c.235 G>A	NSCLC, ESCC	[55,64,65,79]
			Q (Gln)	c.235 G>C	NSCLC, ESCC	[55,63,64,79]
			G (Gly)	c.236 A>G	Larynx	[64]
			E-E	c.234_236 dup AGA	ESCC	[64]
	80	T (Thr)	K (Lys)	c.239 C>A/C>G	NSCLC, ESCC	[55,64,79]
	80	T (Thr)	P (Phe)	c.238 A>C	ESCC	[79]

Domain	Amino acid residue			DNA mutation	Cancer type	References
	locus	Wild Type	Mutant			
			I (Ile)	c.239 C>T	Head and neck	[55]
			A (Ala)	c.238 A>G	NSCLC, hepatoblastoma	[54,71]
	81	G (Gly)	S (Ser)	c.241 G>A	NSCLC, HCC	[65,80]
			V (Val)	c.242 G>T	ESC	[64]
			D (Asp)	c.242 G>A	NSCLC, ESCC	[63,64,79]
	82	E (Glu)	D (Asp)	c.246 A>T	ESCC, oral cancer cell line	[52,55,79]
			G (Gly)	c.245 A>G	NSCLC, PRCC2	[55] [83]
			Q (Gln)	c.244 G>C	NSCLC, ESCC	[63,64]
			V (Val)	c.245 A>T	ESCC	[64]
	83	F (Phe)	L (Leu)	c.247 T>C	NSCLC	[63]

NSCLC, non-small cell lung cancer; ESCC, esophageal squamous cell carcinoma; HCC, hepatocellular carcinoma; PRCC2, type 2 papillary renal cancer. Number of cases - 82 NSCLC and 10 ESCC in [79]; 125 NSCLC, 70 ESCC, 23 larynx, and 17 skin in [64]; 103 NSCLC and 12 head and neck in [55]; 90 NSCLC in [71]; 103 NSCLC in [63]. 5 PRCC2 in [83]; 115 squamous cell cervical carcinomas in [52]; 262 NSCLC in [65]; 48 HCC in [80]; 66 head and neck/tongue squamous cell carcinoma in [81]; 113 ESCC in [53]; 34 primary liver cancer in [68]. Mutation loci in DLG/ETGF not listed for an ESCC (n = 113, [53]) and NSCLC (n = 178, cohorts from The Cancer genome Atlas projects [58], for a Chinese NSCLC cohort from an International Cancer Genome Consortium project (n = 158, [66]), and for an 24 HCC cohort [67].