Genetic variation and gene expression in antioxidant related enzymes and risk of COPD: a systematic review

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ABSTRACT

Background: Observational epidemiological studies of dietary antioxidant intake, serum antioxidant concentration and lung outcomes suggest that lower levels of antioxidant defences are associated with decreased lung function. Another approach to understanding the role of oxidant/antioxidant imbalance in the risk of chronic obstructive pulmonary disease (COPD) is to investigate the role of genetic variation in antioxidant enzymes, and indeed family based studies suggest a heritable component to lung disease. Many studies of the genes encoding antioxidant enzymes have considered COPD or COPD related outcomes, and a systematic review is needed to summarise the evidence to date, and to provide insights for further research.

Methods: Genetic association studies of antioxidant enzymes and COPD/COPD related traits, and comparative gene expression studies with disease or smoking as the exposure were systematically identified and reviewed. Antioxidant enzymes considered included enzymes involved in glutathione metabolism, in the thioredoxin system, superoxide dismutases (SOD) and catalase.

Results: A total of 29 genetic association and 15 comparative gene expression studies met the inclusion criteria. The strongest and most consistent effects were in the genes *GCL*, *GSTM1*, *GSTP1* and *SOD3*. This review also highlights the lack of studies for genes of interest, particularly *GSR*, *GGT* and those related to *TXN*. There were limited opportunities to evaluate the contribution of a gene to disease risk through synthesis of results from different study designs, as the majority of studies considered either association of sequence variants with disease or effect of disease on gene expression.

Conclusion: Network driven approaches that consider potential interaction between and among genes, smoke exposure and antioxidant intake are needed to fully characterise the role of oxidant/antioxidant balance in pathogenesis.

Chronic obstructive pulmonary disease (COPD) is characterised by the development of airflow limitation that is not fully reversible. COPD is a major, and growing, public health burden.[1]

Smoking is the most important risk factor for COPD; however, there is considerable variation in the response to smoke exposure,[2] and it has been estimated that only 15% of the variation in lung function is explained by smoking parameters.[3] While not discounting the paramount importance of smoke exposure in the development of COPD, clearly other factors are significant. Genetic variation is a prime candidate, and many recent studies explore the contribution of genetic variation to interindividual differences in the response to cigarette smoke.

This review focuses on genes related to antioxidant activity, as oxidant-rich cigarette smoke strains the antioxidant defences of the lungs, leading to direct tissue damage and contributing to the inflammation and antiprotease inactivation seen in COPD. This hypothesis is supported by epidemiological studies associating low dietary antioxidant intake and serum antioxidant concentration with decreased lung function[4–9] and increased COPD mortality risk.[10]

Many genetic association studies and comparative expression studies investigated the relation between genes coding for antioxidant enzymes and either COPD or associated traits. An overview of the evidence is warranted to ascertain whether the pattern of published results suggests directions for future research, or whether there are apparent gaps that need to be addressed. Both genetic association studies and gene expression studies were included: polymorphisms can affect disease risk in ways that may or may not be mediated by changes in expression, and expression studies can provide a snapshot of the adaptive response to an exposure. Thus we conducted a systematic review of the literature to characterise the evidence that genes coding for antioxidant enzymes contribute to the aetiology of COPD and related traits.

METHODS

The selection of genes was based mainly on delineating important proteins and the networks of genes that may influence the amount or function of those proteins (fig 1). As glutathione (GSH) is an antioxidant that plays a significant role in the lung, genes encoding GSH associated enzymes were selected. Thioredoxin, which reduces oxidised glutathione and has an antioxidant function that overlaps GSH function, was included with genes encoding associated enzymes. Catalase and superoxide dismutase, two classical antioxidant enzymes of the lung, were also selected. Searches of PubMed were performed up to August 2007 (further details are available online). Published papers considering gene–disease association or differential gene expression in adult humans were selected. Association studies were included if the outcome was disease or lung function. Expression studies were included if the experimental exposure was disease status or smoking and if expression was measured in pulmonary tissues or cells.

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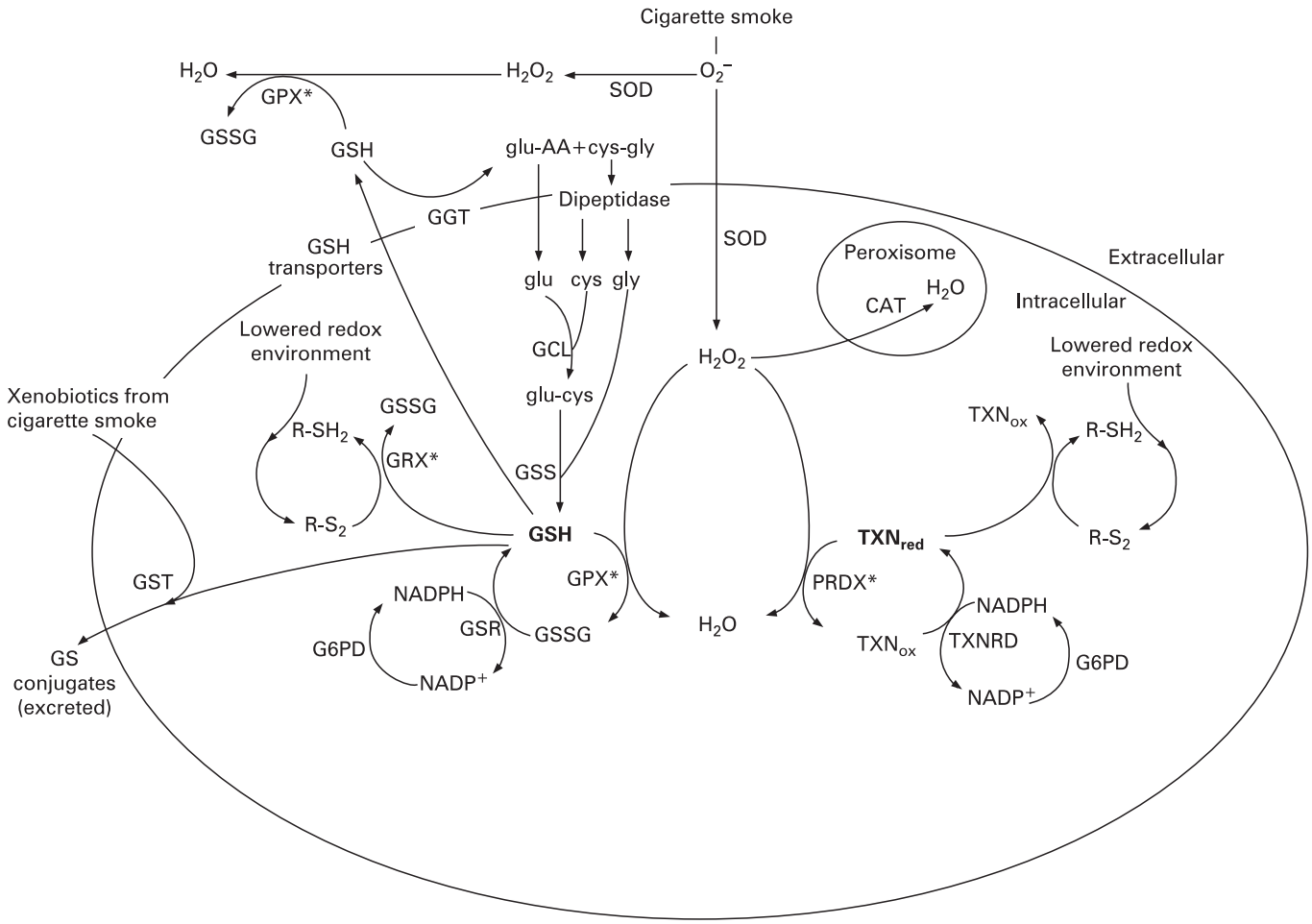

Figure 1 Interaction of antioxidant enzymes in response to oxidative stress. *This reaction also occurs without the listed enzyme. CAT, catalase; cys, cysteine; G6PD, glucose-6-phosphate dehydrogenase; GCL, glutamate–cysteine ligase; GGT, gamma-glutamyl transferase; glu, glutamate; gly, glycine; GPX, glutathione peroxidase; GRX, glutaredoxin; GSH, reduced glutathione; GSR, glutathione reductase; GSS, glutathione synthetase; GSSG, oxidised glutathione; GST, glutathione S-transferase; $NADP^+$, oxidised nicotinamide adenine dinucleotide phosphate; NADPH, reduced nicotinamide adenine dinucleotide phosphate; PRDX, peroxiredoxin; $R-S_2$, disulphide (could be mixed disulphides or glutathionylated proteins); $R-SH_2$, reduced thiol; SOD, superoxide dismutase; TXN_{ox}, oxidised thioredoxin; TXN_{red}, reduced thioredoxin; TXNRD, thioredoxin reductase.

RESULTS

A total of 29 genetic association studies and 14 expression studies were identified (see supplementary table 1 online).

Glutathione synthesis

Three enzymes that relate to glutathione synthesis were considered: gamma-glutamyl transpeptidase (GGT, no studies found), glutamate–cysteine ligase (GCL) and glutathione synthetase (GSS). A substitution in the promoter region of *GCLM* (the modulatory subunit of GCL), leading to decreased glutathione levels,[11] was associated with a threefold increased risk of COPD in Chinese smokers.[12] In the single study of a substitution in the catalytic subunit of GCL (GCLC) that results in decreased expression,[13] an increased prevalence was observed in patients compared with healthy controls (odds ratio (OR) 1.83, 95% confidence interval (CI) 1.00 to 3.36)).[14]

Expression studies of glutathione synthesis compared expression in patients with COPD with asymptomatic smokers and/or non-smokers. Eight of the nine comparisons of *GCLC* expression in lung epithelium found upregulation with disease.[15–18] Two comparisons of *GCLM* expression in lung epithelium of patients with lung tumour with and without COPD showed decreased *GCLM* expression with disease,[17] while the single study of patients with COPD without lung tumour found upregulation.[18]

There were four comparisons of *GCL* expression in lung epithelium of asymptomatic smokers and non-smokers: expression of both subunits was increased in smokers who were healthy volunteers[18 19] and unchanged or decreased in smokers with lung tumour.[17]

Expression of the *GCL* subunits in alveolar macrophages/inflammatory cells showed a more consistent pattern. Both *GCLC*[16 17 20] and *GCLM*[20] were increased in patients with COPD compared with smokers, and smokers had lower expression of both subunits compared with non-smokers.[17 20] A 27% upregulation of the mRNA of *GSS*, the final step of GSH synthesis, was reported in smokers compared with non-smokers ($p = 0.08$),[19] but was not replicated in comparisons with non-smokers with either asymptomatic smokers or patients with COPD.[18 19]

Antioxidant activity of GSH and recycling

Glutathione peroxidase (GPX), glutaredoxin (GLRX), glutathione reductase (GSR) and glucose-6-phosphate dehydrogenase (G6PD) were considered for their role in the antioxidant

activity of GSH and in GSH recycling. There were no association studies of variants in these genes.

Two studies evaluated expression in patients with COPD compared with healthy controls. In the single study of *GPX2*, it was strongly upregulated in patients with COPD at all stages compared with non-smokers (and modestly upregulated compared with smokers).[18] *GPX3* was upregulated in patients with COPD compared with non-smokers, although this difference was not seen in comparison with asymptomatic smokers.[18 21] There was little evidence of differential regulation of *GPX4*, *GPX5* or *GPX7* by disease status.[18]

GPX2 showed a 3–5-fold upregulation in epithelial cells of smokers compared with non-smokers.[18 19] Each of the four studies of *GPX3* expression in epithelial cells reported upregulation in smokers: two studies found a twofold difference[19 22] and a study of alveolar macrophages reported similar differences.[22]

Two expression studies evaluated regulation of *GLRX*. A statistically significant downregulation was observed in the tissue homogenate of patients with COPD compared with smokers (patients had either resection for lung tumour or lung transplantation for severe COPD),[23] but similar results were not observed in an analysis of bronchial epithelial cells.[18] A statistically significant upregulation of GLRX in the sputum of patients with COPD in exacerbation was reported compared with non-smokers.[23]

The sole comparison of GSR expression in epithelial cells by disease groups showed upregulation in patients with COPD.[18] In the two comparisons by smoking status, upregulation was observed among smokers.[18 19] Very similar results were found in the three studies of *G6PD* expression: twofold upregulation in epithelial cells[18 19] and in alveolar macrophages.[24]

In agreement with biological networks, the four expression studies that considered *GPX* (*GPX1*, *GPX2* and *GPX3*), *GSR* and *G6PD* all showed upregulation of each of these genes with smoking.

GSH conjugation and export

There were 24 association and four expression studies of glutathione S-transferases (GSTs), which play a role in GSH conjugation and export.

Homozygous deletion of *GSTM1*, resulting in a complete lack of activity,[25] was associated with increased COPD risk in three of seven association studies (range in OR 2.2 to 8.0).[26–28] The prevalence of the deletion was increased in patients with emphysema compared with non-diseased participants,[29 30] although no association was observed with emphysematous changes in heavy smokers.[31] Both studies of chronic bronchitis reported a threefold increased risk associated with the null genotype.[32 33]

Five studies investigated the association of the *GSTM1* deletion with COPD related quantitative traits. Conflicting evidence was reported: one of two studies of the rate of decline of forced expiratory volume in 1 s (FEV_1) reported an association in men only[34] and one of three studies of FEV_1% predicted reported lower lung function with the null genotype.[35] In a single study of forced vital capacity (FVC) % predicted, the null genotype was significantly associated with decreased lung function.[35] However, it was not associated with an increased rate of FVC decline.[34] The null genotype was associated with a steeper rate of forced expiratory flow $(FEF)_{25-75}$ decline (among men) in one study.[34]

In *GSTP1*, the Ile105Val substitution, which causes altered affinity for specific substrates,[36] was associated with COPD related outcomes. A protective effect of the heterozygous genotype was reported in seven of 11 studies of patients with COPD compared with asymptomatic participants; the magnitude of the effect varied and was statistically significant in two studies.[37 38] A study of smokers with emphysematous changes (compared with normal smokers) reported a protective effect of heterozygosity.[31] However, seven of 10 studies of the homozygous variant genotype in relation to COPD risk reported an increased risk: the difference was statistically significant in one study[39] and four estimates were based on small numbers.

The Ile105Val genotype had little or no relation to the rate of decline in FEV_1, although the direction of effect was consistent with the hypothesis: risk was increased in those homozygous for the variant allele.[34] Greater effect sizes were found for risk of being in the tails of the FEV_1% predicted distribution,[40] but there was little or no continuous relation with FEV_1% predicted.[41]

A *GSTP1* polymorphism with unknown biological effect (Ala114Val) was investigated in three studies. One study of Indian smokers observed a statistically significant graded increase in the prevalence of COPD with the variant allele,[39] but a similar association with emphysema risk was not observed in an American population.[30] In three comparisons of lung function within disease groups, statistically significantly lower lung function was observed with the variant allele in patients with COPD,[39] but not in patients with emphysema[41] or asymptomatic smokers.[39]

There was little or no association of a homozygous deletion of *GSTT1* with COPD risk; three of four studies reported a slightly decreased risk of disease with the null genotype, but the interval estimates of the effect were wide. There was no association of *GSTT1* null and risk of emphysematous changes in smokers.[31] Three of four studies of lung function reported an association: the null genotype was associated with a steeper decline in FEV_1 in a general population,[42] with steeper decline in FEV_1, FVC and FEF_{25-75} among men,[34] and with an increased risk of being in the lowest compared with the highest group of %predicted FEV_1.[40] The only study of mGST1 found no association between four markers and FEV_1 % predicted.[41]

There were three studies of gene expression differences by disease group. Upregulation of *GSTM3* and *mGST1* expression was observed in patients with COPD.[18] *GSTO1* was significantly downregulated in the single study of lung tissue and sputum from patients with COPD with lung tumour,[43] but was upregulated in the epithelial cells of patients with COPD only (less severe stages).[18] Four studies investigated the expression of *GSTs* by smoking status. Both studies of *GSTA1* expression showed upregulation among smokers in lung tissue.[18 21] Statistically significant upregulation was associated with smoking in a single study of *GSTA2*.[19] *GSTM3* expression was increased among smokers to the same extent (approximately 50%) in both studies of epithelial cells.[18 19] There was some evidence of upregulation of *mGST1* among smokers in two studies.[18 19] In six of seven comparisons of *GSTO1* expression in various lung tissues, expression was unrelated to smoking.

Thioredoxin metabolism

Thioredoxin metabolism was evaluated by considering the enzymes thioredoxin (TXN), thioredoxin reductase (TXNRD) and peroxiredoxin (PRDX). No association studies and two expression studies were found. A single study considering epithelial cell expression in COPD compared with non-diseased reported upregulation of *TXN*, *TXNRD1* and *PRDX1* with disease and downregulation of *PRDX5* with disease.[18] Both studies of expression by smoking status reported increased

expression of *TXN* and *TXNRD1* with smoking.[18 19] In the study that also evaluated peroxiredoxins, *PRDX1* was upregulated and *PRDX3* and *PRDX5* were both downregulated in smokers.[18]

Other enzymes

Two classic antioxidants, superoxide dismutase (SOD) and catalase (CAT), were considered. Three association studies (evaluating five variants) and six expression studies were identified.

There was no association between an intronic single nucleotide polymorphism SNP in *SOD1* and COPD. *SOD2* Val16Ala was associated with disease in a Chinese population,[44] but not in persons of European descent.[45] The association between *SOD3* Arg213Gly and COPD was studied in two large populations.[45 46] Heterozygosity was associated with a strong, statistically significant decreased risk of disease (~40–75% reduction)[45 46] and a 70% reduction in risk of COPD hospitalisation or death during follow-up.[46] Genotype was not associated with lung function in a general population, but FEV_1/FVC ratio was higher among smokers with the heterozygous genotype ($p = 0.04$).[46] There were no homozygous variants among diseased individuals in either study, precluding odds ratio calculation.

Three studies of SOD expression compared patients with COPD with healthy controls. SOD activity was increased in the bronchial lavage fluid of non-smokers with COPD compared with smokers with COPD and healthy controls.[47] No evidence of differential expression by disease status was seen in three studies of SOD1.[18 21 48] In the six comparisons of *SOD2* in patients with lung tumour with COPD versus controls, COPD was associated with increased *SOD2* concentration.[21 48] An increase in expression was not observed in the single study of patients with COPD without lung tumour.[18] Neither of the studies of *SOD3* expression in disease groups provided strong evidence for differential regulation by disease.[18 21]

Little or no evidence of differential expression of *SOD1* and *SOD3* by smoking status was observed. *SOD2* was upregulated in smokers compared with non-smokers in three comparisons of epithelial cells (with one showing strong, significant upregulation) and downregulated in three other comparisons. *SOD2* was also upregulated in lung tissue homogenate and alveolar macrophages, but downregulated in the pulmonary blood vessels of smokers compared with non-smokers.

Two studies evaluated polymorphisms in *CAT*, but provided no strong evidence for an association of two promoter region single nucleotide polymorphisms with disease risk.[44 45] Two studies compared *CAT* expression in disease groups, with little evidence for differential regulation by COPD status, although statistically significant downregulation was observed in patients with lung tumour with COPD in one study.[21] Three studies compared *CAT* expression by smoking status, with inconsistent results.

Gene–gene interaction

Increased risk of COPD, and decreased FEV_1 % predicted among patients with COPD, was reported for various genotype combinations that included either *GSTP1* 105Val or 114Val compared with wild-type for both polymorphisms (OR 1.99 for COPD risk; 95% CI 1.28 to 3.09).[39] In an analysis of *GSTM1* null, *GSTT1* null and *GSTP1* Ile105Val, most combinations of the "higher risk" genotypes were associated with an increased risk of disease, with the strongest associations observed with the *GSTP1* 105Ile allele and the null genotype for either *GSTM1* (OR 11.3; 95% CI 1.3 to 98.6) or *GSTT1* (OR 12.1; 95% CI 1.3 to 116.96).[37] Although the combination of *GSTM1* null, *GSTT1* null and *GSTP1* 105Ile was not associated with COPD risk in another study,[49] it was associated with steeper lung function decline ($p = 0.026$)[40] and risk of rapid decline in lung function (OR 2.83; 95% CI 1.1 to 7.2).[42] Men with the null genotype for both *GSTM1* and *GSTT1* had 8.3 ml/year greater decline in FEV_1 compared with those with at least one copy of both ($p<0.001$), with similar results reported for both FVC and FEF_{25-75}.[34] The National Emphysema Treatment Trial reported little or no association between combinations of *GSTM1* null and *GSTP1* 105Ile and disease.[30] There was little or no association of *GSTM1* and *GSTT1* null genotypes and emphysematous changes in Japanese heavy smokers.[31] The combination of *GSTM1* null, *GSTP1* 105 Ile/Ile and at least one slow allele for *microsomal epoxide hydrolase* increased the risk of COPD (OR 6.8; 95% CI 1.6 to 17.2).[26] The combination of *GSTM1* null and a *matrix metalloproteinase 9* polymorphism increased the risk of COPD by about eightfold (OR 7.7; 95% CI 1.1 to 53.3).[27]

DISCUSSION

Observational epidemiological evidence suggests a role for nutrients contributing to antioxidant function in the prevention of lung disease.[4–9] Whether these findings reflect underlying biological mechanisms or methodological bias (eg, confounding) is unclear. Consideration of genetic variants that affect antioxidant/oxidant balance and that may be sensitive to dietary intake of antioxidants can help address this question. The study of genetic variants affecting antioxidant capacity allows an unbiased approach, in comparison with observational studies of diet, based in part on the principles of Mendelian randomisation.[50] Thus this review was designed to evaluate the evidence that antioxidant enzyme function and/or regulation is related to COPD risk.

This systematic review included studies that addressed gene–disease associations as well as those that evaluated differences in gene expression. There were limited opportunities to synthesise results from both approaches as genes were often considered *either* in association studies *or* in expression studies, yet such synthesis may reveal complementary data.[51] For example, a variant allele that leads to decreased glutathione was associated with an increased risk of COPD among smokers,[12] and an expression study of *GCLM* reported upregulation in smokers.[19] The combined results support the hypothesis that increasing available glutathione in persons with a high oxidant load may protect against lung damage. Lack of agreement between association and gene expression studies may also be informative. While association studies suggest a protective effect of heterozygosity for the *GSTP1* Ile105Val polymorphism in COPD, no differences in expression of *GSTP1* were reported in smokers compared with non-smokers, suggesting that the effect of the genotype is not mediated through mRNA quantity.

Comparisons with animal studies provide an additional context in which to interpret the findings from human studies, but caution is warranted. Mice and adult rats (in contrast with humans) synthesise ascorbate,[52] which protects GSH from oxidation and reduces it from its disulphide form.[53] The interaction between ascorbate and other antioxidants suggests that animal studies of genetic manipulation or oxidant insult may not be predictive of results in humans. While two human studies of *GSR* expression reported significantly increased mRNA expression in the airway epithelial cells of asymptomatic smokers,[18 19] findings in smoke exposed rats were mainly negative.[54–56] Reduction of GSSG by endogenously formed

ascorbate may blunt the rat's need for GSR to perform the same function.

This review reveals very few instances where the evidence base contains enough information to make a strong statement of effect, but a few examples deserve mention. The *GSTM1* null genotype (no enzyme activity) was consistently associated with increased COPD risk.[26–29 32–35 37 57] A substitution in another GST, *GSTP1* Ile105Val, which affects catalytic activity and binding affinity for particular substrates, was consistently inversely associated with disease.[31 34 37 38 40 58–60] A rare substitution in *SOD3*, which increases plasma SOD levels, was also associated with a significantly decreased COPD risk,[45 46] a result supported by an animal study: transgenic mice overexpressing human *SOD3* had attenuated lung damage and inflammatory response in hyperoxic conditions.[61] In addition, there was simultaneous upregulation of *GSR*, *GPX* and *G6PD* in the airway epithelial cells of smokers,[19] highlighting the importance of a network of genes in the response of the lung to oxidative stress.

Several elements of the selected interrelated pathways have received minimal or no attention in human studies to date. For example, targeted disruption of the TXN gene produced early embryo lethality in mice,[62 63] and transgenic mice overexpressing human *TXN1* had increased survival and decreased hydroxyl radical production during exposure to diesel exhaust particles.[64] The two studies of TXN related enzymes in humans reported upregulation of *TXNRD1* and *TXN* in the airway epithelial cells of smokers and those with COPD[18 19]: further investigation is warranted. Other understudied genes of interest include *GGT*, *PRDX6* and *GLRX*. GGT is the key enzyme in one pathway for the intracellular supply of cysteine for GSH synthesis: *GGT* deficient mice show a reduced ability for de novo synthesis[65] and decreased intracellular GSH concentration.[66] Furthermore, pulmonary *GGT* activity was increased during hyperoxia in rats,[67] and *GGT* deficient mice had worse survival in hyperoxic conditions.[65 66] PRDX6 is a peroxiredoxin that uses GSH as a cofactor. *PRDX6* null mice had more severe lung injury and significantly decreased survival in conditions of hyperoxia compared with wild-type.[68] Transgenic mice overexpressing *PRDX6* had greater survival and attenuated lung damage in hyperoxia.[69] Finally, GLRX comprises part of a major thiol disulphide redox buffer in the cell. The activity of this enzyme suggests a possible role in relation to the oxidation of GSH and the redox status of the cell, recommending it for further study.

Several methodological considerations deserve mention. COPD aetiology is expected to include gene–environment interactions, given the clear role of smoking in this disease and the interindividual differences in response to cigarette smoke. Thus comparison groups must be carefully selected with regard to smoke exposure. In association studies in which the non-diseased group is comprised of non-smokers and the diseased group of smokers, for instance, the estimate of a true effect may be diluted. Similarly, in expression studies, a comparison between individuals with equivalent exposures, but whose disease outcome differed, may be most informative. Studies published in other languages were included to avoid the "Tower of Babel error".[70] A comprehensive set of enzymes based on biological networks were the starting point for the review, however our selections may have led to inadvertent omission of relevant enzymes. Disturbances in a broad range of redox related enzymes are likely to affect disease risk, suggesting that complex interactions cannot be ignored. A broader network approach may ultimately lead to more robust epidemiological findings.

In conclusion, the evidence summarised in this review supports the continued investigation of the hypothesis that variation in genes that code for enzymes that can alter the redox environment of the lungs may contribute to COPD risk. Future directions suggested by this summary are: more network driven approaches that include broader consideration of enzymes whose related, redundant and linked activities might alter disease risk, further integration of association and expression studies to determine the nature of the biological relationships that may lead to disease, and careful consideration, in both study design and analysis, of environmental exposures (eg, smoking and nutritional status) that are likely to modify the gene–disease associations.

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