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MOLECULAR GENETIC STUDIES OF OXIDATIVE STRESS RELATED GENES

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In memory of Felicitas Maria Therese

ABSTRACT

This work has focused on the basal characterisation of transcriptional regulation, including genetic variants, in oxidative stress related genes and the possible impact of such variation in cardiovascular disease. The genes under study were catalase and MGST1/PGES where the two former protect from hydrogen peroxide and lipid hydroperoxides respectively and the latter is important in e.g. inflammation.

Genomic DNA from ten individuals was sequenced resulting in the discovery of a promoter polymorphism at position –262 from the transcription start site in the human catalase gene. The C to T exchange was shown by reporter gene analysis and transient transfection to influence promoter activity. EMSA analysis showed that there is also a difference in transcription factor binding between the two variants. Catalase protein content measured in whole blood by Western Blot revealed that individuals carrying the T allele have significantly higher catalase levels. This result is consistent with the stronger promoter activity of this allelic variant.

Regulation of the Catalase gene in humans is still unclear. Deletion analysis of the promoter showed that the sequence between –323 and –134 (from the transcription start site) is required for expression of human catalase in HepG2 cells. This sequence includes several GC and CCAAT boxes. Mutational analysis of these cis-acting elements revealed that one CCAAT box (“4”) was essential for basal expression. EMSA analysis showed that the transcription factor NF-Y binds to this element.

The MAPEG members, MGST1 and PGES share 38% identity at the amino acid level and both genes lack TATA boxes. The MGST1 promoter contains one GC box. Reporter constructs transfected into HepG2 cells showed that the GC box is necessary for basal transcription of MGST1. EMSA analysis showed that Sp1 binds to this GC box. The putative promoter of the PGES gene includes two GC boxes. Mutational analysis confirmed that both of them are functional and transfections in A549 cells showed that they are involved in the basal expression of the gene. The transcription factors Sp1 and Sp3 were shown to bind to the GC boxes using EMSA analysis.

The catalase –262 C>T polymorphism was analysed with regard to its potential impact on MI. 2343 individuals (cases and controls) from the SHEEP material were genotyped using the Taqman assay. There was no influence of the polymorphism on risk for MI in the general population. However diabetic carriers of the T allele had a significantly lower risk (OR=0.46, 95% CI 0.23-0.89) of MI compared to diabetics with the C allele. It appears that higher levels of catalase are protective in a risk

population. In summary the work in this thesis has contributed to the understanding of the basal regulation of oxidative stress related genes and the impact of polymorphism in such genes on transcriptional regulation and disease susceptibility.

LIST OF PUBLICATIONS

This thesis is based on the following original articles, which will be referred to in the text by their Roman numerals. The published articles are printed with permission.

I. Forsberg L, **Lyrenäs L**, de Faire U, Morgenstern R. **A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels.**
Free Radic Biol Med. 2001 Mar 1;30(5):500-5.

II. Ekström L, **Lyrenäs L**, Jakobsson PJ, Morgenstern R, Kelner MJ. **Basal expression of the human MAPEG members microsomal glutathione transferase 1 and prostaglandin E synthase genes is mediated by Sp1 and Sp3.**
Biochim Biophys Acta. 2003 Jun 19;1627(2-3):79-84.

III. **Lyrenäs L**, Hägnan C, Morgenstern, R, Ekström L. **Basal expression of the human catalase gene in HepG2 cells is mediated by NF-Y.**
Manuscript (2005)

IV. **Lyrenäs L**, Bennet AM, de Faire U, Morgenstern R. **Catalase gene –262 C>T polymorphism influences the risk of myocardial infarction in diabetic Swedes.**
Manuscript (2005)

PUBLICATIONS NOT INCLUDED IN THE THESIS

Lyrenäs, L., Zotova, E., Ekström, L. and Morgenstern, R.: **Oxidative stress, genetic variation and disease.** In: Oxidative stress, genetic variation and diseaseQuek, J. (Ed.). World Scientific Publishing Co., 2005. InPress.

Lyrenäs, L., Wincent, E., Forsberg, L. and Morgenstern, R.: **The Potential Impact of Polymorphism on Oxidative Stress Status.** In: Redox-Genome Interactions in Health and Disease. Cadenas, E. (Ed.),
Marcel Dekker, Inc., New York, 2004.

Bennet AM, Prince JA, Fei GZ, **Lyrenäs L**, Huang Y, Wiman B, Frostegard J, Faire U. **Interleukin-6 serum levels and genotypes influence the risk for myocardial infarction.** Atherosclerosis. 2003 Dec;171(2):359-67.

CONTENTS

LIST OF ABBREVIATIONS

AP 1	Activator Protein 1
CAT	Catalase
EMSA	Electromobility shift assay
FLAP	5-lipoxygenase-activating protein
GPX	Glutathione peroxidase
GSH	Glutathione
LD	Linkage disequilibrium
LDL	Low density lipoprotein
LTC_4S	Leukotriene C_4 synthase
MAPEG	Membrane associated proteins in Eicosanoid and glutathione metabolism
MGST	Microsomal glutathione S transferase
MI	Myocardial Infarction
NFκB	Nuclear Factor κB
NF-Y	Nuclear Factor Y
PGES	Prostaglandin E synthase
PMA	Phorbol 12-Myristate 13-Acetate
ROS	Reactive oxygen species
SHEEP	Stockholm Heart Epidemiology Programe
SNP	Single nucleotide polymorphism
Sp	Specificity protein
SOD	Superoxide dismutase
TBP	TATA binding protein

INTRODUCTION

This work has focused on basal characterisation of transcriptional regulation including genetic variants in oxidative stress related genes (i.e. catalase and MGST1/PGES) and the possible impact of such variation in cardiovascular disease (i.e. catalase). The introduction gives an overview of molecular genetics, gene regulation, oxidative stress and includes a description of some common oxidative stress related genes and their disease association. Special focus is then directed to introducing the gene families investigated here. For extensive reviews of oxidative stress, human genetic variation and disease the reader is referred to two book chapters by Lyrenäs et al (Lyrenäs et al., 2004; Lyrenäs et al., 2005).

MOLECULAR GENETICS

Molecular genetics is the study of the physics and the chemistry of inheritance. It is primarily concerned with the relationship between the information macromolecules DNA and RNA (that our genes comprise of/code for) and how these molecules are used to synthesise polypeptides, the basic component of all proteins (Strachan and Read, 1999). Our DNA comprises of four building blocks, the bases adenine (A), guanine (G), cytosine (C) and thymine (T) that together in different combinations are the code for the genes in all, living organisms. The genes determine what traits will pass to the next generation and how. They provide detailed instructions to the cells on what to do and when, most often by production of a particular protein.

Molecular genetics is also used to analyse genotype-phenotype relationships. Many diseases are caused by changes in the DNA sequence (e.g. SNPs) of a gene. If the information provided by the gene is altered this can change the protein produced so that it will be more or less effective or not produced at all. In order to study the basal expression of genes as well as the influence of genetic variation on gene expression and pathogenesis there are a wide variety of different techniques such as reporter genes, cell transfections, transformations, EMSAs and methods to study genetic variation.

SNP

90% of the genetic variation found in DNA comprise of single nucleotide polymorphisms (SNPs) (Collins et al., 1998). An SNP is defined as a single base pair

substitution where the allele frequency is at least 1% (Brookes, 1999). Short insertions and deletions (indels) are also considered to be SNPs. It is estimated that 1/500 bp differ between two individuals. About 2/3 of all SNPs are C to T transitions. SNPs can occur in non-coding regions (introns, 5´and 3´UTR), where they can affect RNA stability and splicing. SNPs in the coding regions can result in amino acid exchanges. There are more than 4 million SNPs available in SNP databases (dbSNP). SNPs are used as genetic markers to study the risk of major common diseases such as cancer, cardiovascular disease, diabetes, mental illness and autoimmune states (Brookes, 1999). These variants are also important when studying interindividual differences in drug response to therapeutic drugs. Another important feature of SNPs is their relative stability to mutation, making them useful as markers in linkage disequilibrium (LD) analyses (Forsberg, 2000).

SNP based association studies

Most common diseases are complex disorders involving both genetic components and environmental factors. These types of diseases can be difficult to study relying only on molecular genetic techniques. Association studies involve the comparison of the risk factors for a disease (e.g. SNPs) in affected (patients) and unaffected (matched controls). The validity of this test depends on sample size and an appropriate selection of cases and controls. The selection of the SNPs involved is also important. They should be found in genes relevant to the biology of the disease in question. Preferably results should be repeated in several different populations as disease aetiology can vary.

GENE REGULATION IN HUMAN CELLS

The control mechanisms used to regulate human gene expression are fundamentally similar to those found in other mammals, and resemble those in eukaryotes in general. Gene expression is mostly controlled at three different levels 1) the transcriptional level, which is the major level at which gene expression is controlled 2) the post-transcriptional level and by 3) epigenetic mechanisms. Transcriptional regulation involves histone modification and chromatin remodelling, binding of tissue-specific transcription factors, direct binding of hormones, growth factors or intermediates to response elements in inducible genes and the use of alternative promoters. Post-transcriptional regulation includes alternative splicing, alternative polyadenylation, tissue-specific RNA editing and translational control mechanisms. Epigenetic mechanisms and long-range control of gene expression encompasses allelic exclusion, long-range control by chromatin structure and cell-dependant short-range signalling (Strachan and Read, 1999).

Regulation of transcription

The transcription takes place mostly in the cell nucleus. All genes that encode a protein are transcribed by RNA polymerase II. RNA polymerases cannot initiate transcription by themselves. In order to start the transcription they require the guidance of transcription factors. The transcription factors are protein factors that bind to the DNA and work as recognition signals and activators for the polymerase. As the protein factors engaged in regulating gene expression are themselves encoded by distantly located genes, they are required to migrate to their site of action, and so are called trans-acting factors. In contrast, the regulatory sequences to which they bind are on the same DNA or RNA molecule as the gene or RNA transcript that is being regulated. Such sequences are said to be cis-acting. The promoter is a group of transcription factor binding sites located together upstream of the coding sequence.

Regulation of expression can occur through the core promoter of a gene, at the level of recruitment and processivity of the relevant RNA polymerase. Expression of genes is initiated by the binding of transcription factors to the promoter. Basal levels of transcription can be modulated by binding of protein factors to other regulatory regions occurring in the sequences flanking the gene or sometimes within introns of the gene. There are three cis-acting elements that are especially important for

initiation of transcription by RNA polymerase II. These are the TATA box, the GC box and the CCAAT box.

TATA box

A typical core promoter includes a TATA box (consensus sequence TATA(A/T)A(A/T)). The TATA box is usually located 25-30 bp upstream from the transcription start site. It is the recognition site for the TATA-binding protein (TBP). TBP is a subunit of TFIID, which is part of the general transcription machinery required for transcription.

GC box

These elements are often found in multiple copies located within 100bp of the transcription start site. GC boxes (consensus sequence GGGCGG) are common regulatory elements in all types of human promoters and often regulate transcription in TATA-less genes. Transcriptional regulation by GC boxes is often associated with housekeeping, viral and inducible genes. A CCT repeat has also been shown to function as a GC box (Qin and Rosenfield, 2005). The GC boxes are recognized by the transcription factor family Sp (Specificity protein). There are four members of the Sp family (Sp1-Sp4). Sp1 was originally identified as the transcription factor that bound to and activated transcription in the simian 40 (SV40) promoter (Dynan and Tjian, 1983; Gidoni et al., 1984) and the thymidine kinase promoter (Jones et al., 1985). Sp1 and Sp3 are ubiquitously expressed in mammalian cells. They are structurally similar with similar affinities for the binding site, however their regulatory functions are very different (Li et al., 2004). Sp1 is generally thought to activate transcription while Sp3 works as a suppressor but the opposite has also been observed. The abundance of the Sp1/Sp3 transcription factors and their relative levels are thought to regulate gene expression (Li et al., 2004). Sp1 has been reported to work with other transcription factors such as SREBP (Sterol Response Element Binding Protein), AP1 (Activator Protein 1) and NFκB (Nuclear Factor κB). High levels of Sp4 are found in the brain, where it is believed to exert regulatory functions (Suske, 1999). Sp2 has a different consensus binding site (GGGGTGGGG) and evolutionary background than the other Sp members (Li et al., 2004).

CCAAT box

CCAAT boxes (consensus sequence GGCCAATCT) have been shown to mediate initiation and direction of transcription. It is one of the most common cis-elements being present in about 30% of eukaryotic promoters (Bucher, 1990). It is especially common in cell cycle regulated promoters. The CCAAT box is found in a single copy element in the forward or reverse orientation between –57 and –215 of the major transcription start site, in both TATA-containing and TATA-less promoters (Bucher, 1990; Mantovani, 1999b). CCAAT boxes are extremely conserved within the same gene across species, in terms of position and orientation as well as in the nucleotide flanking the central CCAAT pentanucleotide (Mantovani, 1999b). Analysis of the NF-Y binding sites indicates that mutations of each nucleotide of the CCAAT sequence in a promoter decreases or abolishes NF-Y binding. There are many CCAAT box recognising proteins (e.g. C/EBP, CTF/NF1, CDP). Most of these proteins often do not contain a complete CCAAT binding motif (Maity and de Crombrugghe, 1998). The major recognition protein, which is NF-Y, requires all five nucleotides.

NF-Y was first identified as the activity binding to the MHC class II conserved Y box (Mantovani, 1998). NF-Y is comprised of three subunits; NF-YA, NF-YB and NF-YC, all of them which are necessary for DNA-binding (Sinha et al., 1995). NF-YB contains a histone-fold motif, a TBP-binding domain and is homologous to H2B. NF-YC is similar to NF-YB but has an additional C-treminal Q-rich region and is homologous to H2A (Matuoka and Chen, 2002). NF-YB and NF-YC form a dimer via their histone-fold motifs, which NF-YA can bind to via its interaction domain. The resulting trimer can then bind to DNA with high specificity and affinity (Mantovani, 1999a). Upon binding to the DNA, NF-Y recognises the minor groove and bends the strands in a similar way to the DNA distortion by histone proteins for the nucleosome assembly. This causes local disruption of the nucleosome and, if sufficient, can make room for the factors needed to initiate transcription. NF-Y can increase the affinity of a neighbouring factor for DNA.

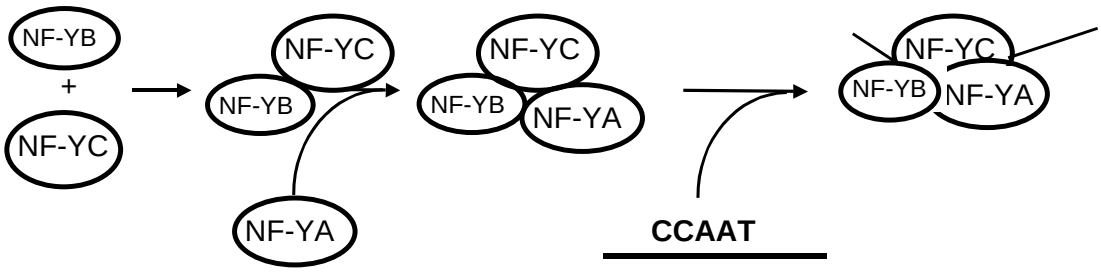

Figure 1. Association of NF-Y subunits and binding to DNA (adapted from Mantovani, R.).

It functionally cooperates with various transcription factors and this cooperation requires precise location of the NF-Y binding site with regard to the binding sites of other transcription factors involved. Such transcriptional synergism occurs together with Sp1 (Wright et al., 1995), SREBP1 (Jackson et al., 1998) and RF-X (Regulatory Factor X) (Reith et al., 1994). NF-Y has been shown to both up regulate transcription and down regulate it (Matuoka and Chen, 2002). The factor also has the ability to interact with various TBP-associated factors (TAFs) (Frontini et al., 2002). TBP has been found to bind to NF-YB and NF-YC (Bellorini et al., 1997). NF-YA seems to be the regulatory subunit in the NF-Y complex. Levels of NF-YA vary in different cell types and/or growth conditions. In young human fibroblasts NF-YA and CCAAT binding was abundant but declined in older cells (Chang and Liu, 1994; Good and Chen, 1996). During the maturation of monocytes to macrophages the levels of NF-Y increase dramatically. Intracellular calcium levels influence DNA binding of NF-Y. Both NF-YB and NF-YC conserved domains contain putative histone fold motifs. NF-Y binding sites are extremely overrepresented in promoters. Generally mutations in the CCAAT motifs cause a several-fold decrease in transcription both in vitro and in vivo (Dorn et al., 1987; Maity et al., 1988; Maire et al., 1989; Hasegawa et al., 1996).

Post-transcriptional regulation

Post-transcriptional regulation of gene expression includes RNA splicing (Steinmetz, 1997), mRNA transport, translation, mRNA stability, protein processing, protein targeting, and protein stability. Alternative splicing is a common feature among human genes. It is estimated that 35%-60% of all human genes undergo alternative splicing (Ladd and Cooper, 2002). Alternative splicing gives rise to different mRNA transcripts (Mironov et al., 1999) by different combinations of exons. This can sometimes lead to related but distinct protein isoforms (Strachan and Read, 1999).

Alternative polyadenylation can be the result of alternative splicing often resulting in tissue-specificity.
RNA editing involves insertions or deletions of single nucleotides at the RNA level. RNA editing has not been observed in mammals.

Epigenetic mechanisms

Epigenetic mechanisms are hereditable nongenomic changes that often result in altered chromatin conformation over long distances. DNA methylation is an epigenetic mechanism, which plays an important part in mammalian gene control, acting as a general method of maintaining repression of transcription. Methylation inhibits transcription through binding of methyl-CpG-binding proteins or through the inhibition of specific transcriptional activators. Methylation of the CpG-islands has been linked to tissue-specific expression of genes. The acetylation and deacetylation of histones is a major regulatory mechanism during gene activation and repression. Histone acetylation leads to transcriptional activity. Histones can also be methylated leading to both transcriptional activation and repression depending on the histone methylated. Chromatin remodelling occurs when ATP hydrolysis is used to change the structure of the nucleosome (Narlikar et al., 2002).

OXIDATIVE STRESS

The presence of free radicals in biological materials was discovered less then 50 years ago. These are partially reduced oxygen species that are involved not only in some of the useful physiological processes but also in pathogenesis of many diseases. During the reduction of oxygen to water in mitochondria approximately 1-2 % of total oxygen consumption gives rise to reactive oxygen species (ROS) such as superoxide anion ($O_2^{\circ -}$) and hydrogen peroxide (H_2O_2) (Radi et al., 1991). The third ROS is hydroxyl radical (OH°), which is formed via the Haber-Weiss reaction between $O_2^{\circ -}$ and H_2O_2. ROS are generally formed due to the absorption of radiation (ionizing, visible, UV or thermal), activation of phagocytic cells and biotransformation of exogenous and endogenous compounds in the endoplasmatic reticulum. ROS are capable of inducing lipid peroxidation which may result in altered membrane protein activity, membrane permeability and transport characteristics, inactivating cellular enzymes by directly oxidising critical protein sulfhydryl or amino groups, depolymerise polysaccharides and inducing DNA strand breaks and chromosome

breakage. Each of these events could lead to cell injury and/or death. OH° generally causes lipid peroxidation. The initiation of this chain-reaction is caused when polyunsaturated fatty acids are attacked by free radicals leading to the conversion of fatty acryl side-chains into lipid hydroperoxides. This process can lead to cleavage of fatty acyl chains into different cytotoxic products such as 4-hydroxynonenal. Cells utilise many different mechanisms to protect themselves from the consequences of ROS, including antioxidant molecules such as vitamin E, ascorbic acid, glutathione and antioxidant enzymes such as superoxide dismutases (SOD), glutathione peroxidase (GPX), catalase (CAT), peroxiredoxins (Hofmann et al., 2002) and many more. These antioxidants often work in a way as to complement each other. The SODs present in all cells catalyze the conversion of $O_2^{\circ -}$ to H_2O_2 and O_2. H_2O_2 is deactivated by GPXs and catalase. GPX is the main enzyme for H_2O_2 decomposition under normal conditions whereas catalase is important for cell protection during conditions of high oxidative stress (Cohen and Hochstein, 1963; Nicholls et al., 1972).

The term oxidative stress is used to describe the imbalance between free radicals and antioxidants. Oxidative stress has been implicated in many disease conditions (Halliwell and Gutteridge, 1989) such as cancer, neurodegenerative diseases and cardiovascular disease.

Gene regulation and oxidative stress

Moderate concentrations of intracellular ROS has been shown to influence gene expression and posttranslational modifications of proteins (Sen and Packer, 1996). Nuclear factor (NF) κB and Activator protein (AP) 1 are two transcription factors that have been identified to be regulated by the intracellular redox state. NF-κB and AP-1 are involved in the inducible expression of several genes involved in oxidative stress and cellular response mechanism.

NF-κB is a member of the Rel proteins that are found in an inactive form in the cytosol. Rel/NF-κB proteins can form homo- or heterodimers. Dimerisation is necessary for DNA binding of the activated factor. The inactivated NF-κB is bound to the inhibitor protein IκB. When stimulated by e.g. H_2O_2, IκB is degraded and the NF-κB is translocated to the nucleus were it can bind to the DNA. NF-κB is implicated in the activation of several gene families encoding cytokines, cytokine-

receptors, cell adhesion molecules, acute-phase proteins and growth factors. NF-κB also plays a role in atherogenesis since oxidised LDL (low density lipoprotein) results in the activation of NF-κB (Parhami et al., 1993). Numerous studies have shown that ROS may serve as a common intracellular agent that contribute to the process of NF-κB activation in response to wide range of stimuli (e.g. H_2O_2, phorbol 12-myristate 13-acetate (PMA) and tumour necrosis factor (TNF-α)) (Suzuki et al., 1995). However cells overexpressing catalase were unable to activate NF-κB in response to TNF-α. Cells overexpressing SOD1 cause activation of NF-κB (Sen and Packer, 1996). Inhibition of NF-κB is also seen at high concentrations of GSSG (glutathione disulfide) (Droge et al., 1994). Thioredoxin upregulates DNA binding of NF-κB in vitro. Other reducing thiol agents are also known to influence NF-κB activation (Sen and Packer, 1996).

AP-1 is a mediator of tumour promotion and is involved in disorders of the nervous system. The transcription factor consists of homo- and heterodimers of the basic region-leucine zipper (bZIP) proteins that belong to the Jun (c-Jun, v-Jun, JunB and JunC) and Fos (c-Fos, v-Fos, FosB, Fra1 and Fra2). c-Jun allows c-Fos to regulate gene expression by serving as an anchor that allows the Jun-Fos heterodimer to bind to the DNA (Sen and Packer, 1996). c-Jun and c-Fos have been shown in several studies to become induced by O_2^- and H_2O_2. Oxidative stress caused by ionizing radiation also induces c-jun expression (Sen and Packer, 1996).

It is important to understand the regulation of genes causing increased and decreased oxidative stress (e.g. myeloperoxidase (MPO) and catalase) as well as the redox regulation of other genes (e.g. AP 1, p 53 and NF-κB) not only with regard to disease conditions but also to learn how the human body functions in everyday life.

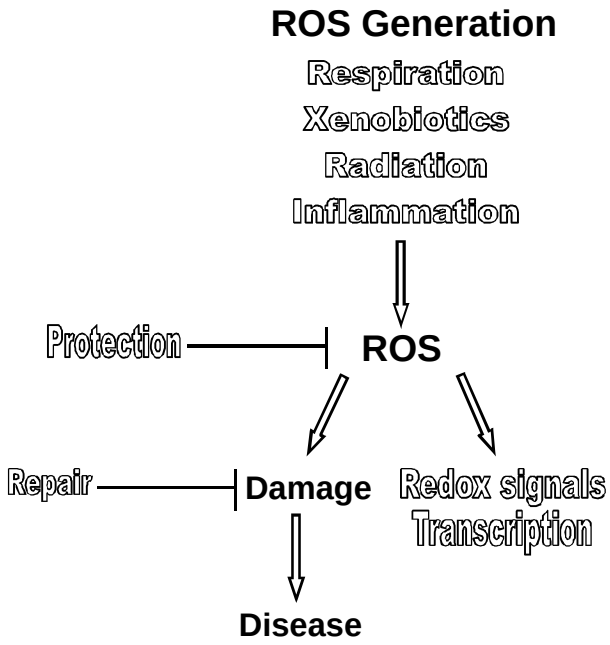

Figure 2. Mulitple levels of genetic influence on ROS damage (indicated by the hollow letters).

HUMAN ANTIOXIDANT ENZYMES

Oxidative stress related genes are implicated in numerous diseases. SNPs can influences the regulation of genes and the production of proteins, which can have an impact on disease. SNP based association studies are thus helpful when studying the relationships between genetic variation, diseases and disease states and environmental factors.

Here is a brief summary of different antioxidant enzymes and antioxidant related genes. Several genetic variants in oxidative stress related genes are attracting considerable interest as tools to understand oxidative stress related disease mechanisms (Forsberg et al., 2001). For a more extensive summary see book chapters by Lyrenäs et al. (Lyrenäs et al., 2004; Lyrenäs et al., 2005).

SUPEROXIDE DISMUTASE

Superoxide dismutases constitute an important antioxidant enzyme defence against reactive oxygen species (ROS) (superoxide anion radicals). At present, three distinct isoforms of SOD have been identified in mammals, and their genomic structure, cDNA, and proteins have been described.

SOD1

Copper/zinc SOD exists as a homodimer and is present in the cytosol of most animal cells. The highest levels are found in liver, erythrocytes, brain and neurons. The gene is located on chromosome 21q22.1 (Huret et al., 1987) and has 5 exons (Orrell et al., 1997). More than 70 different variations have been described in individuals affected by familial amyotrophic lateral sclerosis (ALS) (Rosen et al., 1993; Aoki et al., 1994; Orrell et al., 1997; Gaudette et al., 2000; Liu et al., 2000; Hand et al., 2001; Lindberg et al., 2002; Stathopulos et al., 2003). Variations in *SOD1* were also identified in sporadic cases of Amyotrophic Lateral Sclerosis (ALS) (Gellera et al., 2001; Alexander et al., 2002; Mancuso et al., 2002; Parton et al., 2002; Segovia-Silvestre et al., 2002).

SOD2

Human mitochondrial manganese-containing SOD is a homotetramer located in the mitochondrial matrix. The highest levels of SOD2 can be found in the heart, brain, liver and kidneys (Beyer et al., 1991). The *SOD2* gene is located in the region 6q25.3 and consists five exons (Church et al., 1992). Several genetic variants have been described for the human *SOD2* gene. The substitution of Ala-9Val in the mitochondrial targeting sequence of *SOD2* is associated with an increased risk of sporadic motor neuron disease, especially in females (Van Landeghem et al., 1999). This polymorphism is also associated with nonfamilial idiopathic cardiomyopathy (Hiroi et al., 1999) but has no effect on the occurrence of ALS (Tomblyn et al., 1998; Tomkins et al., 2001) and rheumatoid arthritis in the USA (Mattey et al., 2000). In Japanese patients an association was shown between the Val allele and Parkinson's diseases (PD) (Shimoda-Matsubayashi et al., 1996) but in later studies this association was not confirmed (Grasbon-Frodl et al., 1999; Farin et al., 2001).

SOD3

Extracellular SOD (ECSOD) is a tetrameric glycoprotein containing both copper and zinc. SOD3 is present in plasma, lymph and synovial fluid. This enzyme binds to the heparin sulphate proteoglycans on endothelial surfaces. In humans the highest levels of SOD3 are found in lung, pancreas, thyroid, and uterus (Marklund, 1984). The gene has been mapped to chromosomal region 4p15.3-15.1 (Hendrickson et al., 1990) and contains three exons (Folz et al., 1994). Substitution of arginine in position 213 to glycine causes an 8–15-fold increase in concentration of SOD3 levels in plasma (Folz et al., 1994; Sandstrom et al., 1994; Yamada et al., 1995). The effect of this *SOD3* polymorphism, which has been found in 4% of Swedish (Marklund et al., 1997), 3% of Australian (Adachi et al., 1998) and 6% of Japanese (Folz et al., 1994) subjects, is not entirely clear but early studies suggest that this amino acid variation impairs affinity for heparin and endothelial cell surface and may reduce susceptibility to trypsin-like proteases.

GLUTATHIONE PEROXIDASES

GPX1

Glutathione peroxidases (GPX) catalyze the reduction of organic hydroperoxides, lipid peroxides and hydrogen peroxide, using glutathione as the reducing agent, thereby also protecting cells from oxidative damage resulting from normal oxidative metabolism. There are four known GPXs that contain selenocysteine at the active site.

GPX1 is homotetramer containing one atom of selenium per subunit that metabolizes hydrogen peroxide and a range of organic peroxides, including cholesterol and long-chain fatty acid peroxides (Arthur, 2000).

The *GPX1* gene is located on chromosome 3p21.3 (Kiss, 1997) and contains two exons (Ishida et al., 1987). The enzyme is ubiquitously expressed in humans, most abundantly in liver, erythrocytes and kidneys. Two common polymorphisms have been described for the *GPX1* gene. A proline to leucine substitution at codon 198, resulting from a C>T transition at nucleotide 593 and a (GCG)n repeat polymorphism coding for alanine residues in a polyalanine tract (Moscow JA, 1994). The Pro198Leu polymorphism was significantly associated with the risk of developing lung cancer (Ratnasinghe D, 2000) and breast cancer (Hu YJ, 2003). The Pro and Leu variants of human GPX1 do not differ in activity and stability of enzyme and is not significantly associated with an increased risk for stroke (Forsberg L, 2000).

The (GCG)n repeat polymorphism showed significant association to an increased risk of coronary artery disease (CAD) for individuals with at least one ALA6 allele (Blankenberg S, 2003; Winter JP, 2003) but not with prostate cancer (Kote-Jarai Z, 2002).

GPX2

GPX2 is primarily expressed in the gastrointestinal tissues (Chu et al., 1993) and plays an important role in the protection against ingested lipid peroxides. GPX2 is a cytosolic protein. The gene is located on chromosome 14q24.1 and has two exons. Although two polymorphisms have been reported for this gene there have been no association studies published to date.

GPX3

The extracellular GPX is present mainly in the plasma but also in kidneys, lungs, heart and placenta. The GPX3 gene has been mapped to chromosomal region 5q32.q33.1 and contains five exons (Yoshimura et al., 1994). No polymorphisms of interest have been found for this gene.

GPX4

GPX4 is a monomeric enzyme with mitochondrial and non-mitochondrial forms. GPX4 is highly expressed in testes and thyroid but can be detected in most tissues. This enzyme is capable of reducing peroxidized phospholipids, cholesterol hydroperoxides and thiamine hydroperoxides. Thus, GPX4 is considered to be an important enzymatic defence against oxidation of biomembranes (Kelner and Montoya, 1998). The GPX4 gene has 7 exons and has been mapped to chromosome 19p13.3. In testicular tissue, *GPX4* is expressed in three different forms. The full-length cDNA clone of GPX4 has alternative start sites, which can code for proteins of 197 or 170 amino acids. The differences between the two potential forms are 27 amino acids at the N-terminal region. These 27 amino acids constitute a mitochondrial targeting sequence (Pushpa-Rekha et al., 1995). Recently 23 different variant sites were identified in *GPX4* (Maiorino et al., 2003). Four substitution variants mapped to the promoter region (positions 2221, 2197, 2180, and 2100), and nine mapped to exons, i.e., two in the 5' untranslated region (UTR) of the mRNA, four in the coding region, and three in the 3' UTR of the gene. Only one of the exon variants leads to an Ala93Thr exchange that reduces activity in a porcine GPX4 homologue. Two detected promoter variations were shown by reporter gene constructs to affect transcription in somatic cell lines.

GPX5

GPX5 is the only GPX lacking the seleno-cycteine residue in the catalytic site. GPX 5 expression is only found in the epidimyis (Grignard et al., 2005). The GPX 5 gene has been mapped to chromosome 6p22.1. A 118 nt frame-shift deletion resulting in an inactive protein has been identified as the major transcript (Hall et al., 1998). This deletion makes GPX5 selenium-independent with very little activity towards hydrogen peroxide or organic hydroperoxides. No association studies with GPX5 have been published.

CATALASE

In 1818, Thernard first observed that animal tissues could decompose H_2O_2. In 1901, Loew introduced the name catalase for the specific enzyme that decomposes H_2O_2. In 1923 it was suggested by Warburg that catalase was an iron-containing enzyme because it was inhibited by cyanide. Zeile and Hellström provided evidence that the prosthetic group was haematin in 1930. The main purpose of catalase is to catalyse the decomposition of H_2O_2 to water and oxygen, which it can do by two separate reactions. Both reactions begin with the formation of a primary complex (compound I) between hydrogen peroxide and the iron of the haematin prosthetic group (Deisseroth and Dounce, 1970). In the second reaction Compound I reacts with two electrons that are transferred from an electron donor (e.g. H_2O_2, methanol, ethanol or formic acid) to form water and an oxidised product. The second electron transfer is slow compared to the first and results in an accumulation of the reduced form of compound I called Compound II. Compound II is an inactive form of the enzyme. There is also a Compound III, which is a reversible complex formed when treating Compound II with H_2O_2. The overall reactions of catalase can be summarised as

1) $\text{P-Fe}^{3+}\text{-OH} + H_2O_2 \rightarrow \text{P-Fe}^{3+}\text{-OOH} + H_2O$

2) $\text{P-Fe}^{3+}\text{-OOH} + H_2O_2 \rightarrow \text{P-Fe}^{3+}\text{-OH} + 2H_2O + O_2$

The mechanism of formation and the structures of Compound I, II and III are still not completely understood neither is the mechanism of catalysis by catalase. Catalase also has a role in ethanol metabolism, specifically during high blood alcohol levels (Bradford et al., 1993).

$$CH_3CH_2 + H_2O_2 \xrightarrow{\text{Catalase}} CH_3CHO + H_2O$$

Catalase is a ubiquitous antioxidant enzyme expressed in all aerobic organisms containing a cytochrome system. It can be found in most tissues but is most abundant in liver, kidney and erythrocytes. The lowest levels are expressed in connective tissue

and brain. Mitochondria in most cells lack catalase (Bai and Cederbaum, 2001) with the exception of rat heart (Radi et al., 1991). In the liver, catalase is mostly found in peroxisomes but it has also been identified in the endoplasmic reticulum and cytoplasm. It is found free in the cytosol of mature erythrocytes except for a small part that is bound to the membrane (Ogata, 1991). Its steady-state concentration in erythrocytes has been estimated at 10^{-10} mol/L (Giulivi et al., 1994). Mammalian catalase occurs as a complex of four identical subunits. Each subunit has a molecular weight of approximately 60 kDa and contains a single ferriprotoporphyrin (haematin) group (Quan et al., 1986). In the liver catalase is synthesised as a monomer in the endoplasmic reticulum and is then transferred to the peroxisomes where it is assembled to the tetramer (Percy, 1984). Catalase is encoded by a single-copy gene, which is highly conserved through out evolution (Bell et al., 1986; Quan et al., 1986; Nakashima et al., 1989; Reimer et al., 1994). The gene is located on chromosome 11p13 and spans 34 kb. It consists of 13 exons and 12 introns. The introns range in size from about 400 bp to 10.5 kb (Quan et al., 1986). Human catalase consists of four polypeptide chains each containing 526 amino acid residues (Korneluk et al., 1984; Quan et al., 1986). Although isoforms of catalase have been described in some organisms (e.g. maize) there is no evidence for any isozymes in human tissues (Deisseroth and Dounce, 1970).

The basal regulation of the catalase gene is unclear. It is thought to be transcriptionally regulated in a tissue-specific manner. Several common features of housekeeping genes have been discovered in the 5´-flanking region of catalase genes from various species. One such feature is the lack of a TATA box to regulate transcription and the presence of several GC and CCAAT boxes. In mouse muscle cells, the basal expression of catalase has been shown to be dependant on the binding of the transcription factor NF-Y to two CCAAT boxes (Luo and TA, 2003). In human HP 100 cells, a variation of human leukaemia HL60 cells that over express catalase, the GC box located at position –71 from the ATG was shown to regulate the basal expression. The surrounding CCAAT boxes were shown to function as activators and repressors of transcription (Nenoi et al., 2001). In breast cancer cells catalase has also been shown to be regulated by autocrine human growth hormone (hGH) via the p44/42 MAP kinase pathway. The autocrine production of hHG transcriptionally upregulates catalase mRNA and protein levels, increasing catalase activity (Zhu et al., 2005). The 3´region of the catalase gene is highly A/T rich and the presence of four ATTTA sequences (Quan et al., 1986) suggests the possibility of a regulated

degradation of its transcripts. Catalase can be upregulated by oxidative stress (Hunt et al., 1998). Cells overexpressing catalase have an increased protection against H_2O_2. However catalase overexpressing cells are more vulnerable to damage by radiation. It has also been shown that catalase transcription is activated by panaxadiol (Chang et al., 1999).

Several rare polymorphisms have been found in the *CAT* gene, most of them associated with the catalase-deficiency acatalasemia (see the section on acatalasemia below).

A common C >T exchange has been found at –262 bp from the transcription start site. This SNP alters the level of catalase in blood, influences transcription factor binding and promoter activity (PaperI). This polymorphism has been studied in a number of different association studies. There was no association to Alzheimers disease (Goulas et al., 2002), SLE (Systemic Lupus Erythematosus) in a Korean population (Eny et al., 2005), acute pancreatitis in an English population (Rahman et al., 2005) or age related phenotypes in a Danish population (Christiansen et al., 2004). In an American breast cancer study the T allele was associated with an decreased risk of breast cancer (Ambrosone et al., 2005). The T allele is also associated with a decreased risk of diabetes type I in a Russian population (Chistiakov et al., 2004) and with reduced risks of DPN (Diabetic polyneuropathy) in Diabetes type I patients (Zotova et al., 2004). However, in arsenic-induced hyperkeratosis the T allele is associated with elevated risks of developing disease (Ahsan et al., 2003). No association was found with Diabetes type II in an Hungarian population (Szelestei et al., 2005).

A promoter polymorphism described in a Chinese population (–844 C>T) has been associated with blood pressure levels (Jiang et al., 2001). Also a common T>C polymorphism at nucleotide position 1167 has been described (Forsberg et al., 1999). An uninformative genetic marker has been described in exon 10 a T>C exchange together with a C>T substitution in exon 9 that is associated with vitiligo (Casp et al., 2002). There is no known association between catalase polymorphisms and familial amyotrophic lateral sclerosis (FALS) (Parboosingh et al., 1995).

Acatalasemia

Several rare polymorphisms have been detected in the catalase gene, most of them associated with acatalasemia. Acatalasemia is inherited as an autosomal recessive trait and is characterised by erythrocyte catalase levels 0.2-4% of normal.

Acatalasemic patients do not totally lack catalase. Takahara first described the catalase deficiency in 1948. The reported clinical phenotype is gangrene and atrophy of the mouth. However in recent years no such symptoms have been observed. Acatalasemia is the homozygous catalase-deficient state while hypocatalasemia is the heterozygous condition. Cases of acatalasemia have been reported from 9 countries, with most cases in Japan (91 patients in 46 families) and Switzerland (11 patients in 3 families) (Góth et al., 2000). The frequency of actalasemia has been estimated at 1/25000 for any population and 2-5/1000 for hypocatalasemics (Eaton and Ma, 1995). The Japanese type A acatalasemia is due to a defect in the syntheses of the protein i.e. an abnormal splicing of catalase mRNA due to a guanine to adenine (A→G) transition at the fifth position of intron 4 (Wen et al., 1990; Góth et al., 2000). The abnormal splicing leads to a decrease of catalase activity but no other substantial differences when compared to the normal protein. Japanese type B acatalasemia is due to a deletion of a thymine at position 358 in exon 4. This deletion gives a frameshift mutation and introduces a stop codon, resulting in a truncated protein with no catalase activity (Hirono et al., 1995). Low but significantly raised levels of GPX are found in Japanese acatalasemics (Ogata et al., 1972; Ogata et al., 1977b).

The Swiss-type is caused by a point mutation, resulting in an amino acid substitution (Aebi et al., 1968), (Hirono et al., 1995) which does not influence the amount of mRNA (Crawford et al., 1988). The exact type and location of the mutation is still unclear. The catalase produced is rapidly degraded and has a unique electrophoretic mobility (Aebi et al., 1968). In Japanese actalasemics catalase is deficient in other tissues than erythrocytes (Wen et al., 1990). In the Swiss type of acatalasemia higher levels of catalase were detected in tissues such as liver and reticulocytes (Wen et al., 1990).

In Hungary a catalase deficiency has been described in 13 families (2 acatalasemics and 61 hypocatalasemics). There are four types of Hungarian actalasemia. Type A depends on a guanine and adenine insertion at position 138 in exon 2, resulting in a frameshift and a stopcodon causing truncated protein (Góth et al., 2000). Hungarian type B acatalasemia is caused by a guanine insertion at position 79 in exon 2. This mutation also causes a truncated protein (Eaton and Ma, 1995).Type C is caused by thymine to guanine substitution in intron 7 leading to decreased catalase activity (Goth et al., 2001b). The last type described is type D which is caused by a guanine to adenine exchange in exon 9 position 5, leading to the substitution of the essential amino acids Arginin 354 to cystein 354 (Goth et al., 2005). These four mutations are

responsible for catalase deficiency in 65% of the Hungarians with this condition. Hungarian and Japanese hypocatalsemics have a catalase activity of about 50% compared to normocatalsemics (Ogata et al., 1977a; Goth, 2001) while Swiss hypocatalasemics have almost normal activities (Aebi et al., 1977). Hungarian subjects with catalase deficiency have higher levels of erythrocyte SOD and GPX activitities (Goth and Eaton, 2000). Inherited catalase deficiency seems to be associated with an increased risk of diabetes in the Hungarian population (Goth and Eaton, 2000; Goth et al., 2001a). Significant increase of total cholesterol, LDL cholesterol, ApoA1, ApoB, Lp (a) and a decrease in LDL oxidative resistance has also been observed (Goth, 2000).

THE MAPEG FAMILY

The MAPEG (membrane-associated proteins in eicosanoid and glutathione metabolism) superfamily of structurally and phylogenetically related enzymes, including Microsomal glutathione S-transferase 1 (MGST1), Microsomal glutathione S-transferase 2 (MGST2), Microsomal glutathione S-transferase 3 (MGST3), prostaglandin E synthase (PGES), 5-lipoxygenase activating protein (FLAP), and leukotriene C4 synthase (LTC_4S). Enzymes in this superfamily have distinct or overlapping functions involving detoxification, protection from oxidative stress, glutathione peroxidase activity or synthesis of prostaglandin E and cysteinyl leukotrienes (Jakobsson et al., 1999; Jakobsson et al., 2000).

MGST1

Microsomal Glutathione S-transferase 1 (MGST1) is a trimeric, membrane-bound enzyme that catalyses the conjugation of electrophilic compounds with glutathione and the reduction of lipid hydroperoxides (Mosialou et al., 1993; Andersson et al., 1994; Mosialou et al., 1995). MGST1 has been found in all tissues examined but is most abundant in liver (Estonius et al., 1999). *MGST1* is 18 kb and has seven exons, three comprise the coding region and four are alternatively spliced first exons, where one is predominant. The *MGST1* gene has been mapped to chromosome 12p13.3 (Kelner et al., 1996; Estonius et al., 1999; Lee and DeJong, 1999). The functional promoter of *MGST1* is GC-rich and lacks a TATA box. MGST1 has one GC box located 28 bp from the transcription start site. The GC box has been shown to be

functional and requires binding of Sp1 for basal transcription (Paper II). The functional *MGST1* promoter region responds to oxidative stress induced by paraquat or menadione in HepG2 cells (Kelner et al., 2000).

MGST1 is contains several polymorphisms, 46 SNPs, 4 in the promoter region, 34 in introns, 3 in 3´UTR and five in the 3´flanking region and 13 insertion-deletions have been found in a Japanese population. No SNPs were detected in the 5´UTR or coding regions (Iida A. et al., 2001). (Forsberg et al., 1999) report a T>G substitution in the 3´-flanking region, 2 intronic polymorphisms and an A>G-314 promoter polymorphism. Several polymorphisms have been reported in databases but as of yet potential functional consequences are unknown.

MGST2 and MGST3

MGST2 and MGST3 are glutathione-dependent enzymes and that catalyze the reduction of 5-hydroperoxyeicosatetraenoic acid and conjugation of leukotrienes (LT)A_4 to form LTC_4. Unlike MGST3, MGST2 shows activity with 1-chloro-2, 4-dinitrobenzene (CDNB). MGST2 is found mostly in liver, spleen, skeletal muscle, heart and pancreas (Jakobsson et al., 1996; Jakobsson et al., 1997b) and MGST3 is found in the heart, skeletal muscle and adrenal cortex (Jakobsson et al., 1997a). *MGST2* is located on chromosome 4q28.31 (Jakobsson et al., 1996). *MGST3* is located on chromosome 1q23 (Jakobsson et al., 1997a) and has six exons (Thameem et al., 2003). Three SNPs in *MGST2* have been reported by Iida and collegues (Iida et al., 2001) and Thameem et al. (Thameem et al., 2003) reported 25 variations. However in the databases over a hundred polymorphisms have been reported for both *MGST2* and *MGST3*. *MGST 2* has 195 hits in the SNP database (http://www.ncbi.nlm.nih.gov/SNP/). All but 33 are either validated or genotyped or both. Four amino acid exchanges were found. The dbSNP includes 193 reported SNPs for *MGST 3*. The majority are either validated or genotyped or both. No association studies have been published for MGST2 whereas a MGST3 variant was reported to have no association with diabetes type 2 in Pima Indians (Thameem et al., 2003).

PGES

Prostaglandin E synthase (PGES) was previously known as MGST1L-1. Prostate, testis, bladder and placenta are the tissues in which the highest mRNA levels of PGES have been observed (Jakobsson et al., 1999). The *PGES* gene, consists of three

exons and spans 14.8 kb and is located on chromosome 9q34.3. PGES has a 38% identity to MGST1 on the amino acid sequence level (Jakobsson et al., 1999; Jakobsson et al., 2000). The transcription start site has not yet been identified in the *PGES* gene. However a 171 bp fragment directly upstream of the translation start site exhibit promoter activity when transfected in A549 cells. The putative *PGES* promoter activity was strongly reduced by Phenobarbital (PB), and the PGES protein induction by Interleukin-1β (IL-1β) was also blocked by this agent (Forsberg L, 2000). The putative promoter of *PGES* is GC-rich and lacks a TATA-box. The two GC boxes that are located 103bp and 114bp from translation start site are both functional. It has been shown that both Sp1 and Sp3 are involved in the basal transcription of human PGES (Paper II).

FLAP

The 5-lipoxygenase activating protein (FLAP) is together with 5-lipoxygenase and leukotriene C_4 synthase (LTC_4S) required for leukotriene synthesis. Leukotrienes are arachidonic acid metabolites that have been implicated in various types of inflammatory responses, including asthma, arthritis and psoriasis. The exact function of the FLAP enzyme remains controversial, but previous studies suggest that it acts as an arachidonic acid transfer protein for 5-lipoxygenase. The human *FLAP* gene contains five exons and is located on chromosome 13q12 (Ford-Hutchinson et al., 1994).

Three polymorphisms were found in the coding region, but none of them resulted in an amino acid substitution (In et al., 1997). Two SNPs were detected in the *FLAP* promoter region and an insertion/deletion polymorphism affecting Sp1 and early growth response-1 (Egr-1) transcription factor binding sites. The wild type allele consisted of five Sp1 binding sites repeated in tandem. Reporter gene experiments demonstrated that alleles consisting of a deletion of one or two of these sites or addition of a sixth binding site resulted in reduced gene expression (Drazen and Silverman, 1999; In et al., 1999).

A G>A substitution at -336 bp and a poly (A) repeat (n=19 or 23) at position -169 to -146 bp was identified in the *FLAP* promoter. There was no support for a significant role for these polymorphisms in genetic susceptibility to asthma in the Caucasian population (Sayers et al., 2003).

A four-SNP marker haplotype in this locus spanning the *FLAP* gene is associated with a two times greater risk of myocardial infarction in Iceland (Helgadottir et al., 2004).

LTC_4S

The LTC_4S enzyme catalyzes the GSH-dependent conversion of leukotriene A_4 to leukotriene C_4, potent biological compounds derived from arachidonic acid. LTC_4S is active as a homodimer and is present in eosinophils, basophils, mast cells and macrophages (Lam et al., 1994; Welsch et al., 1994). Leukotrienes have been implicated as mediators of anaphylaxis and inflammatory conditions such as human bronchial asthma. The human *LTC_4S* gene contains four exons and located on chromosome 5q35 (Penrose et al., 1996). LTC_4S and FLAP have the same intron/exon junctions but differ in their UTR sizes.

A SNP consisting of A>C transversion –444 nucleotides upstream of the ATG translation start site in the *LTC4S* gene has been associated with a relative risk for the aspirin-intolerant asthmatic phenotype in Polish patients (Sanak et al., 1997) and in a Japanese population (Kawagishi et al., 2002). No such association could be found in a US population (Van Sambeek et al., 2000). The -444 C allele was associated with higher levels of *LTC_4S* mRNA in eosinophils (Sampson et al., 2000; Sanak and Szczeklik, 2000).

METHODOLOGICAL CONSIDERATIONS

Promoter studies

The most common method used for studying transcriptional regulation is transient transfection assays. It can also be used to study the effect of polymorphisms in promoter sequences. The promoter of interest is introduced into the cells by transfection of artificial gene expression systems into an appropriate cell system. The transfection vectors utilised are designed so that expression of a reporter gene, whose protein can be easily measured, is controlled almost entirely by the sequence of interest. Among the commonly used reporter genes are chloramphenicol acetyltransferase (CAT), β-galactosidase, luciferase from the American firefly and green fluorescent protein (GFP).

The American firefly gene was used for the experiments in papers I, II and III. Gene regulation was also studied by deletion analysis and site-directed mutagenesis. These are techniques that map cis-acting elements, which can control gene expression.

EMSA

Electromobility shift assay (EMSA) is a technique to identify DNA-protein interaction. It distinguishes different DNA-protein complexes by size in a polyacrylamide-gel. The double-stranded, radiolabelled oligonucelotide complementary to the binding site of the trans-acting element is mixed with nuclear extract from appropriate cells. In order to determine if the complexes contain the protein of interest an unlabelled competitor and/or antibodies against the protein of interest can be used. DNA-protein interaction can be studied under more “in vivo” like conditions using the chromatin immunoprecipitation (ChIP) assay. This is a method that preserves the architecture of higher order chromatin structures by cross-linking cells with formaldehyde (Orlando et al., 1997). DNA-protein interactions can be studied in virtually intact chromatin.

Genotyping

There are a great number of genotyping techniques available today, all of the commonly used methods are PCR-based (e.g. hybridisation, mass spectrometry, pyrosequencing). Deciding what method to use usually comes down to cost per

genotyping. Requiring a method which is of low cost, high throughput and reproducible.

Restriction enzyme analysis has been used to determine the allele frequency in Paper I. This is a convenient, small-scale way of allelic discrimination based on the loss or gain of a restriction site. Mismatched primers can be used to create suitable restriction sites when the SNP does not alter the recognition sequence for a restriction enzyme. In Paper IV the fluorogenic 5´- nuclease polymerase chain reaction (TaqMan) was used according to the principle reviewed by Livak et al (Livak, 1999). This is a high through-put method used for real-time quantitive PCR detection. It is a sensitive method with low template usage (2-20 ng) and a closed system, which decreases the risk of contamination. During the Taqman PCR the product accumulation is measured by fluorescence and compared to a standard. The fluorescent signal can be generated by cleavage by a sequence-specific oligonucleotide labelled with a dye and a quencher (Strachan and Read, 1999).

The SHEEP material

An association study involves determining the frequency of a test factor (e.g. SNP) in patients and matched controls. The validity of this test will then depend on the appropriate selection of these patients and controls (Brookes, 1999). The Stockholm Heart Epidemiology program (SHEEP) has been described in detail previously (Reuterwall et al., 1999; Bennet et al., 2002). The total study includes fatal or nonfatal MI cases (n=2246), age 45-70, and their age, sex and residential area matched controls (n=3206). One control per case was randomly selected from the Stockholm county population registry.

The SHEEP study is one of the largest and most comprehensive materials available today for the study of MI. It is used in paper IV to study the impact of the CAT –262 C>T polymorphism on MI.

PRESENT STUDY

AIMS OF THE STUDY

- To study the phenotype of the CAT –262 C>T polymorphism.

- To characterise the promoter of the human catalase gene in terms of basal regulation.

- To characterise the MAPEG-members PGES and MGST1 in terms of basal regulation.

- To study the possible impact of the CAT –262 C>T polymorphism in MI.

RESULTS/DISCUSSION

A common functional C-T substitution polymorphism in the promoter region of the human catalase gene influences transcription factor binding, reporter gene transcription and is correlated to blood catalase levels. (Paper I)

In this paper we investigated the promoter region of the human Catalase gene for genetic variation. Genomic DNA from 10 individuals was screened for polymorphisms in the 5´-flanking region by direct sequence analysis of PCR products. A common C>T polymorphism was found –262 bp from the transcription start site. Computer analysis with MatInspector 2.2 indicated that different transcription factors might bind with different affinities to the two variants. We performed transient transfection with reporter constructs, EMSA and quantified the protein in human blood by Western blot in order to determine if there were any phenotypic consequences associated with this polymorphism.

Reporter constructs containing a 348 bp sequence including either a T or a C at the polymorphic site were constructed using a pGL2 basic vector with a luciferase reporter gene. The constructs were transfected into three different cell types (human hepatoma HepG2 cells, human epithelial cervix HeLa cells and human erythroid K562 cells). Differences in the promoter activity between the two variants could be seen in the HepG2 cells and the K562 cells. No significant differences were however seen in HeLa cells. To further characterise the two variant forms, EMSA was used to study DNA-protein interaction. PMA-stimulated HeLa cells showed altered DNA-protein binding compared to untreated cells. Differences in protein binding were also visible between the two variants using nuclear extracts from K562 cells. Although different transcription factors are clearly involved we were unable to identify them.

Western blot was used to study the relationship between the polymorphism and catalase levels in whole blood. When comparing the individual genotypes, a decreasing trend from TT to CT to CC could be seen. Carriers of the T allele have significantly higher levels of catalase in blood compared with individuals homozougus for the C allele. These results are consistent with the results from the reporter gene experiments. The fact that the promoter polymorphism is associated with an altered phenotype provided an SNP tool for epidemiological association studies.

Basal expression of the human MAPEG members microsomal glutathione transferase 1 and prostaglandin E synthase genes is mediated by Sp1 and Sp3. (Paper II)

In this study we investigated the basal expression of Microsomal Glutathione transferase (MGST1) and Prostaglandin E synthase (PGES), which are both members of the MAPEG (Membrane Associated Proteins involved in Eicosanoid and Glutathione metabolism) superfamily. We characterised the promoter regions and identified the elements required using in vitro assays, including reporter analysis of deletion and mutant clones and EMSA.

5´deletion analysis of a 562 bp pGL3MGST1 construct was performed. Five fragments were subcloned into reporter constructs containing the luciferase gene and transiently transfected into HepG2 cells. The –393 fragment showed the highest activity, indicating that there is a negative regulator(s) between (-393-(-510)). Otherwise sequential deletions from the –393 construct from the 5´-direction gradually reduced reporter activity. The elimination of two HNF1 sites and one GC box decreased MGST1 promoter activity by 45%. The results were not seen in human alveolar A549 cells indicating that HNF1 may play a vital role for the expression of MGST1 in HepG2 cells. The results show that the sequence between –40 and –20 contains the element necessary for the basal transcription. Mutation analysis was used to identify the GC box (28 bp from the transcription start site) as critical for the basal expression of the MGST1 gene. EMSA analysis, using nuclear extract from phorbol stimulated HeLa cells, showed that the transcription factor Sp1 binds to the GC box and is mediating basal transcription of MGST1.

Mutation analysis was used to study the two GC boxes in the 171 bp construct of the putative PGES promoter. Transfection assays with wild type and mutant reporter constructs transfected into human alveolar A549 cells to study the effects of the GC mutations on luciferase activity were performed. Mutation of GC box 1 and 2 reduced activity to 10% and 20 % of wild type controls. The activity was reduced to 15% when both GC boxes were mutated. The results confirm that both GC boxes are functional.

Five DNA-protein complexes were seen in the EMSA, using nuclear extracts from phorbol-stimulated HeLa cells. Results indicate that Sp1 binds to two of the

complexes while Sp3 only binds to one. Indicating that both Sp1 and Sp3 are involved in the basal transcription of PGES.

The PGES promoter has been shown to be down regulated by Phenobarbital (PB). Therefore the two Barbie (Barbituate response elements) were studied by site-directed mutagenesis. Transfection of the wild type and the mutated constructs of a 651 bp pGL3 basic vector in A549 cells showed no difference in response to PB treatment. These results confirm previous studies indicating that the PB-Responsive enhancer Module (PBREM) rather than the Barbie box is the *cis*-acting element involved in PB regulation (Sueyoshi and Negishi, 2001).

Basal expression of human catalase in HepG2 cells is mediated by NF-Y. (Paper III)

In this study we isolated and characterised the human Catalase promoter. Deletion analyses of the promoter subcloned into pGL3 basic vectors with a luciferase gene and then transiently transfected into HepG2 cells revealed that the minimum sequence needed for basal expression resides between –323 and –134. This was defined by the transcriptional activity of pGL3-323 and the lack of activity with pGL3-134. The human catalase promoter contains a total of three GC-boxes and five CCAAT-boxes. All but one of the GC boxes and all the CCAAT boxes are located in this region. The lack of activity in the pGL3-134 construct is not surprising as there are no GC or CCAAT boxes located in that region.

The influence of the GC and CCAAT boxes was studied by site-directed mutagenesis, using the pGL3-T vector and transfection into HepG2 cells. Mutation of GC box 1T had a negative influence on transcription while GC boxes 1C, 2 and 3 had no significant influence at all. These results were confirmed by transfection in Drosophila DSL2 cells with Sp1 and Sp3 expression vectors. Thus GC boxes are not necessary for basal expression of the human catalase gene. The individual CCAAT boxes were also mutated and analysed in HepG2 cells. Although slight variation in expression efficiency could be seen when mutating CCAAT boxes 1,2,3 and 5 the changes were not significant. Mutation of CCAAT box 4 (located at –163 from ATG) completely abolished expression. This indicates that CCAAT box 4 is crucial for the basal expression of the catalase gene. These results confirm previous data obtained with the mouse catalase gene (Luo and TA, 2003) but contradict results from studies on the human catalase gene in HL60 cells (Nenoi et al., 2001) .

DNA-protein interaction was studied by EMSA. Results show that when using nuclear extract from HepG2 cells the transcription factor NF-Y binds to all five CCAAT boxes. Showing that NF-Y plays a role as an activator of human catalase. Expression studies with reporter constructs containing the mutated CCAAT-boxes identified a single CCAAT box (pos.-163 from ATG) that was absolutely required for transcriptional activity. Our results identify the basal regulatory mechanism of human catalase in a relevant cell line, representing an organ (liver) where the enzyme is highly expressed, and forms a foundation for the analysis of polymorphic variants in the gene promoter that influence gene expression.

The Catalase gene –262 C>T polymorphism influences the risk of myocardial infarction in diabetic Swedes. (Paper IV)

Here we study the influence of the common promoter polymorphism –262 C>T in the catalase gene and its possible impact on MI. Oxidative stress has been implicated in several different diseases including cardiovascular disease and therefore variation in protective genes could be hypothesised to be important.

Genomic DNA from 2343 individuals (men and women) included in the SHEEP study were genotyped by the fluorogenic 5´- nuclease polymerase chain reaction (TaqMan) method as described by Livak et al (Livak, 1999). Available for genotyping were 1044 cases (736 men and 308 women) and 1299 controls (870 men and 429 women). The 2343 individuals include cases i.e. first time non-fatal MIs and their age, sex and residential area matched controls. There was generally a difference in occurrence of risk factors between cases and controls. Cases were more exposed to various cardiovascular risk factors (Reuterwall et al., 1999).

Carriers of the T allele were considered exposed (TT and TT/CT) whereas those homozygous for the C allele are defined as unexposed. The observed genotypes were in Hardy-Weinberg equilibrium. There was no significant difference in genotype distribution between cases and controls, also when analysed separately for men and women, indicating that this polymorphism was not related to MI in this Swedish population.

There was however a significant difference in the genotype distribution related to MI when comparing diabetics (n=196) and non-diabetics (n=2147). Diabetic carriers of the T allele had a significant decreased risk of MI (OR=0.46, 95% CI 0.23-0.89). When related to smoking there was a nonsignificant dose-dependant decrease in

protection. Where the T allele protection was strong in never smokers (OR=0.22, 95% CI 0.05-0.10), it decreased in ex-smokers (OR=0.66, 95% CI 0.19-2.35) and was completely abolished in current smokers (OR=1.01, 95% CI 0.25-3.98).

The fact that the CAT –262 C>T polymorphism was protective against MI in diabetics in the Swedish population demonstrates from a molecular genetic perspective, that oxidative stress is contributing to MI disease aetiology in diabetics. The effect of smoking demonstrates that once an additional risk factor (leading to increased oxidative stress) is present the beneficial effect of increased catalase expression is obviated.

CONCLUSIONS

- A promoter polymorphism has been found –262 bp from the transcription start site in the human catalase gene.

- The polymorphism influences transcription factor binding, promoter activity and blood protein levels.

- The basal expression of MGST1 is influenced by Sp1-binding to the GC box located 28 bp from the transcription start site.

- Both Sp1 and Sp3 are involved in the basal expression of PGES.

- Both GC boxes in PGES have been shown to be functional.

- The Barbie box is not responsible for the downregulation of PGES by PB.

- The basal transcription of human catalase is mediated through NF-Y binding to a CCAAT box (“4”) in HepG2 cells.

- The CAT –262 C>T polymorphism is protective against MI in diabetics in a Swedish population. A role for oxidative stress is thus implicated.

FUTURE PERSPECTIVES

• The understanding of exactly how the –262 C>T polymorphism influences the transcription of the human catalase gene is still unclear. It would be desirable to learn which transcription factors bind to the two variants. This could also shed light on cell/tissue specific differences.

•Finding phenotypically manifested genetic variants also in MGST1/PGES genes.

•There are contradictory data in the literature concerning the relation between the activity of catalase and the genetic variants investigated here (-262C>T). Therefore the molecular basis for discrepancies between catalase levels and activities in blood need to be determined.

• The effect of the catalase -262 C>T polymorphism on MI in diabetics needs to be further explored in a larger sample of diabetics. Is the at risk allele (lower catalase levels) related to more serious forms of diabetes or to a more serious outcome in terms of cardiovascular disease?

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REFERENCES

Adachi, T., Yamazaki, N., Tasaki, H., Toyokawa, T., Yamashita, K. and Hirano, K.: Changes in the heparin affinity of extracellular-superoxide dismutase in patients with coronary artery atherosclerosis. Biol Pharm Bull 21 (1998) 1090-3.

Aebi, H., Bossi, E., Cantz, M., Matsubara, S. and Suter, H.: Acatalasemia. In: Beutler, E. (Ed.), Hereditry disorders of Erythrocyte metabolism. Grune and Stratton, New York, 1968, pp. 41-83.

Aebi, H., Wyss, S.R. and Scherz, B.: Unstable mutants and molecular hybrids in enzyme deficiency conditions. Acta Biol Med Ger 36 (1977) 735-41.

Ahsan, H., Chen, Y., Kibriya, M.G., Islam, M.N., Slavkovich, V.N., Graziano, J.H. and Santella, R.M.: Susceptibility to arsenic-induced hyperkeratosis and oxidative stress genes myeloperoxidase and catalase. Cancer Lett 201 (2003) 57-65.

Alexander, M.D., Traynor, B.J., Miller, N., Corr, B., Frost, E., McQuaid, S., Brett, F.M., Green, A. and Hardiman, O.: "True" sporadic ALS associated with a novel SOD-1 mutation. Ann Neurol 52 (2002) 680-3.

Ambrosone, C.B., Ahn, J., Singh, K.K., Rezaishiraz, H., Furberg, H., Sweeney, C., Coles, B. and Trovato, A.: Polymorphisms in genes related to oxidative stress (MPO, MnSOD, CAT) and survival after treatment for breast cancer. Cancer res 65 (2005) 1105-1111.

Andersson, C., Mosialou, E., Weinander, R. and Morgenstern, R.: Enzymology of microsomal glutathione S-transferase. Adv Pharmacol 27 (1994) 19-35.

Aoki, M., Ogasawara, M., Matsubara, Y., Narisawa, K., Nakamura, S., Itoyama, Y. and Abe, K.: Familial amyotrophic lateral sclerosis (ALS) in Japan associated with H46R mutation in Cu/Zn superoxide dismutase gene: a possible new subtype of familial ALS. J Neurol Sci 126 (1994) 77-83.

Arthur: The glutathione peroxidases. Cell. Mol. Life Sci. 57 (2000) 1825-1835.

Bai, J. and Cederbaum, A.I.: Mitochondrial catalase and oxidative injury. Biol Signals Recept 10 (2001) 189-99.

Bell, G.I., Najarian, R.C., Mullenbach, G.T. and Hallewell, R.A.: cDNA sequence coding for human kidney catalase. Nucleic Acids Res 14 (1986) 5561-2.

Bellorini, M., Lee, D.K., Dantonel, J.C., Zemzoumi, K., Roeder, R.G., Tora, L. and Mantovani, R.: CCAAT binding NF-Y-TBP interactions: NF-YB and NF-YC require short domains adjacent to their histone fold motifs for association with TBP basic residues. Nucleic Acids Res 25 (1997) 2174-81.

Bennet, A.M., Brismar, K., Hallqvist, J., Reuterwall, C. and De Faire, U.: The risk of myocardial infarction is enhanced by a synergistic interaction between serum insulin and smoking. Eur J Endocrinol 147 (2002) 641-7.

Beyer, W., Imlay, J. and Fridovich, I.: Superoxide dismutases. Prog Nucleic Acid Res Mol Biol 40 (1991) 221-53.

Blankenberg S, R.H., Bickel C, Torzewski M, Hafner G, Tiret L, Smieja M, Cambien F, Meyer J, Lackner KJ;: Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. N Engl J Med. 349(17) (2003) 1605-13.

Bradford, B.U., Seed, C.B., Handler, J.A., Forman, D.T. and Thurman, R.G.: Evidence that catalase is a major pathway of ethanol oxidation in vivo: dose-response studies in deer mice using methanol as a selective substrate. Arch Biochem Biophys 303 (1993) 172-6.

Brookes, A.J.: The essence of SNPs. Gene 234 (1999) 177-86.

Bucher, P.: Weight matrix descriptions of four eukaryotic RNA polymerase II promoter elements derived from 502 unrelated promoter sequences. J Mol Biol 212 (1990) 563-78.

Casp, C.B., She, J.X. and McCormack, W.T.: Genetic association of the catalase gene (CAT) with vitiligo susceptibility. Pigment Cell Res 15 (2002) 62-6.

Chang, M.S., Lee, S.G. and Rho, H.M.: Transcriptional activation of Cu/Zn superoxide dismutase and catalase genes by panaxadiol ginsenosides extracted from Panax ginseng. Phytother Res 13 (1999) 641-4.

Chang, Z.F. and Liu, C.J.: Human thymidine kinase CCAAT-binding protein is NF-Y, whose A subunit expression is serum-dependent in human IMR-90 diploid fibroblasts. J Biol Chem 269 (1994) 17893-8.
Chistiakov, D.A., Savost'anov, K.V., Turakulov, R.I., Titovich, E.V., Zilberman, L.I., Kuraeva, T.L., Dedov, II and Nosikov, V.V.: A new type 1 diabetes susceptibility locus containing the catalase gene (chromosome 11p13) in a Russian population. Diabetes Metab Res Rev 20 (2004) 219-24.
Christiansen, L., Petersen, H.C., Bathum, L., Frederiksen, H., McGue, M. and K., C.: The Catalase -262 C/T promoter polymorphism and aging phenotypes. Journal of Gerontology 59A (2004) 886-889.
Chu, F.F., Doroshow, J.H. and Esworthy, R.S.: Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. J Biol Chem 268 (1993) 2571-6.
Church, S.L., Grant, J.W., Meese, E.U. and Trent, J.M.: Sublocalization of the gene encoding manganese superoxide dismutase (MnSOD/SOD2) to 6q25 by fluorescence in situ hybridization and somatic cell hybrid mapping. Genomics 14 (1992) 823-5.
Cohen, G. and Hochstein, P.: Glutathione Peroxidase: The Primary Agent for the Elimination of Hydrogen Peroxide in Erythrocytes. Biochemistry 172 (1963) 1420-8.
Collins, F.S., Brooks, L.D. and Chakravarti, A.: A DNA polymorphism discovery resource for research on human genetic variation. Genome Res 8 (1998) 1229-31.
Crawford, D.R., Mirault, M.E., Moret, R., Zbinden, I. and Cerutti, P.A.: Molecular defect in human acatalasia fibroblasts. Biochem Biophys Res Commun 153 (1988) 59-66.
Deisseroth, A. and Dounce, A.L.: Catalase: Physical and chemical properties, mechanism of catalysis, and physiological role. Physiol Rev 50 (1970) 319-75.
Dorn, A., Bollekens, J., Staub, A., Benoist, C. and Mathis, D.: A multiplicity of CCAAT box-binding proteins. Cell 50 (1987) 863-72.
Drazen, J.M. and Silverman, E.S.: Genetic determinants of 5-lipoxygenase transcription. Int Arch Allergy Immunol 118 (1999) 275-8.
Droge, W., Schulze-Osthoff, K., Mihm, S., Galter, D., Schenk, H., Eck, H.P., Roth, S. and Gmunder, H.: Functions of glutathione and glutathione disulfide in immunology and immunopathology. Faseb J 8 (1994) 1131-8.
Dynan, W.S. and Tjian, R.: The promoter-specific transcription factor Sp1 binds to upstream sequences in the SV40 early promoter. Cell 35 (1983) 79-87.
Eaton, J.W. and Ma, M.: Actalasemia, 2nd ed. McGraw Hill, New York, 1995.
Eny, K.M., El-Sohemy, A., Cornelis, M.C., Sung, Y.K. and Bae, S.C.: Catalase and PPARgamma2 genotype and risk of systemic lupus erythematosus in Koreans. Lupus 14 (2005) 351-5.
Estonius, M., Forsberg, L., Danielsson, O., Weinander, R., Kelner, M.J. and Morgenstern, R.: Distribution of microsomal glutathione transferase 1 in mammalian tissues. A predominant alternate first exon in human tissues. Eur J Biochem 260 (1999) 409-13.
Farin, F.M., Hitosis, Y., Hallagan, S.E., Kushleika, J., Woods, J.S., Janssen, P.S., Smith-Weller, T., Franklin, G.M., Swanson, P.D. and Checkoway, H.: Genetic polymorphisms of superoxide dismutase in Parkinson's disease. Mov Disord 16 (2001) 705-7.
Folz, R.J., Peno-Green, L. and Crapo, J.D.: Identification of a homozygous missense mutation (Arg to Gly) in the critical binding region of the human EC-SOD gene (SOD3) and its association with dramatically increased serum enzyme levels. Hum Mol Genet 3 (1994) 2251-4.
Ford-Hutchinson, A.W., Gresser, M. and Young, R.N.: 5-Lipoxygenase. Annu Rev Biochem 63 (1994) 383-417.
Forsberg, L.: Genetic variation and regulation of oxidative stress related genes, Institutet of Enviromental medicine. Karolinska Institutet, Stockholm, 2000.
Forsberg, L., de Faire, U. and Morgenstern, R.: Low yield of polymorphisms from EST blast searching: analysis of genes related to oxidative stress and verification of the P197L polymorphism in GPX1. Hum Mutat 13 (1999) 294-300.

Forsberg, L., de Faire, U. and Morgenstern, R.: Oxidative stress, human genetic variation, and disease [Review]. Archives of Biochemistry & Biophysics 389 (2001) 84-93.
Forsberg L, d.F.U., Marklund SL, Andersson PM, Stegmayr B, Morgenstern R. . Phenotype determination of a common Pro-Leu polymorphism in human glutathione peroxidase 1. Blood Cells Mol Dis. Oct;26(5): (2000) 423-6.
Frontini, M., Imbriano, C., diSilvio, A., Bell, B., Bogni, A., Romier, C., Moras, D., Tora, L., Davidson, I. and Mantovani, R.: NF-Y recruitment of TFIID, multiple interactions with histone fold TAF(II)s. J Biol Chem 277 (2002) 5841-8.
Gaudette, M., Hirano, M. and Siddique, T.: Current status of SOD1 mutations in familial amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 1 (2000) 83-9.
Gellera, C., Castellotti, B., Riggio, M.C., Silani, V., Morandi, L., Testa, D., Casali, C., Taroni, F., Di Donato, S., Zeviani, M. and Mariotti, C.: Superoxide dismutase gene mutations in Italian patients with familial and sporadic amyotrophic lateral sclerosis: identification of three novel missense mutations. Neuromuscul Disord 11 (2001) 404-10.
Gidoni, D., Dynan, W.S. and Tjian, R.: Multiple specific contacts between a mammalian transcription factor and its cognate promoters. Nature 312 (1984) 409-13.
Giulivi, C., Hochstein, P. and Davies, K.J.: Hydrogen peroxide production by red blood cells. Free Radic Biol Med 16 (1994) 123-9.
Good, L.F. and Chen, K.Y.: Cell cycle- and age-dependent transcriptional regulation of human thymidine kinase gene: the role of NF-Y in the CBP/tk binding complex. Biol Signals 5 (1996) 163-9.
Goth, L.: Lipid and carbohydrate metabolism in acatalasemia. Clin Chem 46 (2000) 564-6.
Goth, L.: A new type of inherited catalase deficiencies: its characterization and comparison to the Japanese and Swiss type of acatalasemia. Blood Cells Mol Dis 27 (2001) 512-7.
Goth, L. and Eaton, J.W.: Hereditary catalase deficiencies and increased risk of diabetes. Lancet 356 (2000) 1820-1.
Goth, L., Lenkey, A. and Bigler, W.N.: Blood catalase deficiency and diabetes in Hungary. Diabetes Care 24 (2001a) 1839-40.
Goth, L., Rass, P. and Madarasi, I.: A novel catalase mutation detected by polymerase chain reaction-single strand conformation polymorphism, nucleotide sequencing, and western blot analyses is responsible for the type C of Hungarian acatalasemia. Electrophoresis 22 (2001b) 49-51.
Góth, L., Shemirani, A. and Kalmár, T.: A novel catalase mutation (a GA insertion) causes the hungarian type of acatalasemia. Blood cells, molecules and disease 26 (2000) 151-154.
Goth, L., Vitai, M., Rass, P., Sukei, E. and Pay, A.: Detection of a novel familial catalase mutation (Hungarian type D) and the possible risk of inherited catalase deficiency for diabetes mellitus. Electrophoresis 26 (2005) 1646-9.
Goulas, A., Fidani, L., Kotsis, A., Mirtsou, V., Petersen, R.C., Tangalos, E. and Hardy, J.: An association study of a functional catalase gene polymorphism, -262C-->T, and patients with Alzheimer's disease. Neurosci Lett 330 (2002) 210-3.
Grasbon-Frodl, E.M., Kosel, S., Riess, O., Muller, U., Mehraein, P. and Graeber, M.B.: Analysis of mitochondrial targeting sequence and coding region polymorphisms of the manganese superoxide dismutase gene in German Parkinson disease patients. Biochem Biophys Res Commun 255 (1999) 749-52.
Grignard, E., Morin, J., Vernet, P. and Drevet, J.R.: GPX5 orthologs of the mouse epididymis-restricted and sperm-bound selenium-independent glutathione peroxidase are not expressed with the same quantitative and spatial characteristics in large domestic animals. Theriogenology 64 (2005) 1016-33.
Hall, L., Williams, K., Perry, A.C., Frayne, J. and Jury, J.A.: The majority of human glutathione peroxidase type 5 (GPX5) transcripts are incorrectly spliced: implications for the role of GPX5 in the male reproductive tract. Biochem J 333 (Pt 1) (1998) 5-9.
Halliwell, B. and Gutteridge, J. Clarendon press, Oxford, 1989.

Hand, C.K., Mayeux-Portas, V., Khoris, J., Briolotti, V., Clavelou, P., Camu, W. and Rouleau, G.A.: Compound heterozygous D90A and D96N SOD1 mutations in a recessive amyotrophic lateral sclerosis family. Ann Neurol 49 (2001) 267-71.
Hasegawa, T., Zhou, X., Garrett, L.A., Ruteshouser, E.C., Maity, S.N. and de Crombrugghe, B.: Evidence for three major transcription activation elements in the proximal mouse proalpha2(I) collagen promoter. Nucleic Acids Res 24 (1996) 3253-60.
Helgadottir, A., Manolescu, A., Thorleifsson, G., Gretarsdottir, S., Jonsdottir, H., Thorsteinsdottir, U., Samani, N.J., Gudmundsson, G., Grant, S.F., Thorgeirsson, G., Sveinbjornsdottir, S., Valdimarsson, E.M., Matthiasson, S.E., Johannsson, H., Gudmundsdottir, O., Gurney, M.E., Sainz, J., Thorhallsdottir, M., Andresdottir, M., Frigge, M.L., Topol, E.J., Kong, A., Gudnason, V., Hakonarson, H., Gulcher, J.R. and Stefansson, K.: The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke. Nat Genet 36 (2004) 233-9.
Hendrickson, D.J., Fisher, J.H., Jones, C. and Ho, Y.S.: Regional localization of human extracellular superoxide dismutase gene to 4pter-q21. Genomics 8 (1990) 736-8.
Hiroi, S., Harada, H., Nishi, H., Satoh, M., Nagai, R. and Kimura, A.: Polymorphisms in the SOD2 and HLA-DRB1 genes are associated with nonfamilial idiopathic dilated cardiomyopathy in Japanese. Biochem Biophys Res Commun 261 (1999) 332-9.
Hirono, A., Sasaya-Hamada, F., Kanno, H., Fuji, H., Yoshida , T. and Miwa, S.: A novel human catalase mutation (358 T-del) causing Japanese type acatalasemia. Blood Cell. Mol. Dis. 21 (1995) 232-233.
Hofmann, B., Hecht, H.J. and Flohe, L.: Peroxiredoxins. Biol Chem 383 (2002) 347-64.
Hu YJ, D.A.: Role of glutathione peroxidase 1 in breast cancer: loss of heterozygosity and allelic differences in the response to selenium. Cancer Res. 63(12) (2003) 3347-51.
Hunt, C.R., Sim, J.E., Sullivan, S.J., Featherstone, T., Golden, W., Von Kapp-Herr, C., Hock, R.A., Gomez, R.A., Parsian, A.J. and Spitz, D.R.: Genomic instability and catalase gene amplification induced by chronic exposure to oxidative stress. Cancer Res 58 (1998) 3986-92.
Huret, J.L., Delabar, J.M., Marlhens, F., Aurias, A., Nicole, A., Berthier, M., Tanzer, J. and Sinet, P.M.: Down syndrome with duplication of a region of chromosome 21 containing the CuZn superoxide dismutase gene without detectable karyotypic abnormality. Hum Genet 75 (1987) 251-7.
Iida, A., Saito, S., Sekine, A., Kitamoto, T., Kitamura, Y., Mishima, C., Osawa, S., Kondo, K., Harigae, S. and Nakamura, Y.: Catalog of 434 single-nucleotide polymorphisms (SNPs) in genes of the alcohol dehydrogenase, glutathione S-transferase, and nicotinamide adenine dinucleotide, reduced (NADH) ubiquinone oxidoreductase families. J Hum Genet 46 (2001) 385-407.
Iida A., Saito S. and Sekine A.: Catalog of 46 single-nucleotide polymorphisms (SNPs) in the microsomal glutathione S-transferase 1 (MGST1) gene. J Hum Genet 46 (2001) 590-594.
In, K.H., Asano, K., Beier, D., Grobholz, J., Finn, P.W., Silverman, E.K., Silverman, E.S., Collins, T., Fischer, A.R., Keith, T.P., Serino, K., Kim, S.W., De Sanctis, G.T., Yandava, C., Pillari, A., Rubin, P., Kemp, J., Israel, E., Busse, W., Ledford, D., Murray, J.J., Segal, A., Tinkleman, D. and Drazen, J.M.: Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. J Clin Invest 99 (1997) 1130-7.
In, K.H., Silverman, E.S., Asano, K., Beier, D., Fischer, A.R., Keith, T.P., Serino, K., Yandava, C., De Sanctis, G.T. and Drazen, J.M.: Mutations in the human 5-lipoxygenase gene. Clin Rev Allergy Immunol 17 (1999) 59-69.
Ishida, K., Morino, T., Takagi, K. and Sukenaga, Y.: Nucleotide sequence of a human gene for glutathione peroxidase. Nucleic Acids Res 15 (1987) 10051.

Jackson, S.M., Ericsson, J., Mantovani, R. and Edwards, P.A.: Synergistic activation of transcription by nuclear factor Y and sterol regulatory element binding protein. J Lipid Res 39 (1998) 767-76.
Jakobsson, P.J., Mancini, J.A. and Ford-Hutchinson, A.W.: Identification and characterization of a novel human microsomal glutathione S-transferase with leukotriene C4 synthase activity and significant sequence identity to 5-lipoxygenase-activating protein and leukotriene C4 synthase. J Biol Chem 271 (1996) 22203-10.
Jakobsson, P.J., Mancini, J.A., Riendeau, D. and Ford-Hutchinson, A.W.: Identification and characterization of a novel microsomal enzyme with glutathione-dependent transferase and peroxidase activities. J Biol Chem 272 (1997a) 22934-9.
Jakobsson, P.J., Morgenstern, R., Mancini, J., Ford-Hutchinson, A. and Persson, B.: Common structural features of MAPEG -- a widespread superfamily of membrane associated proteins with highly divergent functions in eicosanoid and glutathione metabolism. Protein Sci 8 (1999) 689-92.
Jakobsson, P.J., Morgenstern, R., Mancini, J., Ford-Hutchinson, A. and Persson, B.: Membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG). A widespread protein superfamily. Am J Respir Crit Care Med 161 (2000) S20-4.
Jakobsson, P.J., Scoggan, K.A., Yergey, J., Mancini, J.A. and Ford-Hutchinson, A.W.: Characterization of microsomal GST-II by western blot and identification of a novel LTC4 isomer. J Lipid Mediat Cell Signal 17 (1997b) 15-9.
Jiang, Z., Akey, J.M., Shi, J., Xiong, M., Chen, H., Wu, H., Xiao, J., Lu, D., Huang, W. and Jin, L.: A polymorphism in the promoter region of catalase is associated with blood pressure levels. Hum Genet 109 (2001) 95-98.
Jones, D.P., Eklow, L., Thor, H. and Orrenius, S.: Metabolism of hydrogen peroxide in isolated hepatocytes: relative contributions of catalase and glutathione peroxidase in decomposition of endogenously generated H2O2. Arch Biochem Biophys 210 (1981) 505-16.
Jones, K.A., Yamamoto, K.R. and Tjian, R.: Two distinct transcription factors bind to the HSV thymidine kinase promoter in vitro. Cell 42 (1985) 559-72.
Kawagishi, Y., Mita, H., Taniguchi, M., Maruyama, M., Oosaki, R., Higashi, N., Kashii, T., Kobayashi, M. and Akiyama, K.: Leukotriene C4 synthase promoter polymorphism in Japanese patients with aspirin-induced asthma. J Allergy Clin Immunol 109 (2002) 936-42.
Kelner, M.J., Bagnell, R.D., Montoya, M.A. and Lanham, K.A.: Structural organization of the human gastrointestinal glutathione peroxidase (GPX2) promoter and 3'-nontranscribed region: transcriptional response to exogenous redox agents. Gene 248 (2000) 109-16.
Kelner, M.J. and Montoya, M.A.: Structural organization of the human selenium-dependent phospholipid hydroperoxide glutathione peroxidase gene (GPX4): chromosomal localization to 19p13.3. Biochem Biophys Res Commun 249 (1998) 53-5.
Kelner, M.J., Stokely, M.N., Stovall, N.E. and Montoya, M.A.: Structural organization of the human microsomal glutathione S-transferase gene (GST12). Genomics 36 (1996) 100-3.
Kiss, C.L., J.; Szeles, A.; Gizatullin, R. Z.; Kashuba, V. I.; Lushnikova, T.; Protopopov, A. I.; Kelve, M.; Kiss, H.; Kholodnyuk, I. D.; Imreh, S.; Klein, G.; Zabarovsky, E. R.: Assignment of the ARHA and GPX1 genes to human chromosome bands 3p21.3 by in situ hybridization and with somatic cell hybrids. Cytogenet. Cell Genet 79 (1997) 228-230.
Korneluk, R.G., Quan, F., Lewis, W.H., Guise, K.S., Willard, H.F., Holmes, M.T. and Gravel, R.A.: Isolation of human fibroblast catalase cDNA clones. Sequence of clones derived from spliced and unspliced mRNA. J Biol Chem 259 (1984) 13819-23.
Kote-Jarai Z, D.F., Edwards SM, Hamoudi R, Jackson RA, Ardern-Jones A, Murkin A, Dearnaley DP, Kirby R, Houlston R, Easton DF, Eeles R.: Association between the GCG polymorphism of the selenium dependent GPX1 gene and the risk of young onset prostate cancer. Prostate Cancer Prostatic Dis. 5(3) (2002) 189-92.

Ladd, A.N. and Cooper, T.A.: Finding signals that regulate alternative splicing in the post-genomic era. Genome Biol 3 (2002) reviews0008.
Lam, B.K., Penrose, J.F., Freeman, G.J. and Austen, K.F.: Expression cloning of a cDNA for human leukotriene C4 synthase, an integral membrane protein conjugating reduced glutathione to leukotriene A4. Proc Natl Acad Sci U S A 91 (1994) 7663-7.
Lee, S.H. and DeJong, J.: Microsomal GST-I: genomic organization, expression, and alternative splicing of the human gene. Biochim Biophys Acta 1446 (1999) 389-96.
Li, L., He, S., Sun, J.M. and Davie, J.R.: Gene regulation by Sp1 and Sp3. Biochem Cell Biol 82 (2004) 460-71.
Lindberg, M.J., Tibell, L. and Oliveberg, M.: Common denominator of Cu/Zn superoxide dismutase mutants associated with amyotrophic lateral sclerosis: decreased stability of the apo state. Proc Natl Acad Sci U S A 99 (2002) 16607-12.
Liu, H., Zhu, H., Eggers, D.K., Nersissian, A.M., Faull, K.F., Goto, J.J., Ai, J., Sanders-Loehr, J., Gralla, E.B. and Valentine, J.S.: Copper(2+) binding to the surface residue cysteine 111 of His46Arg human copper-zinc superoxide dismutase, a familial amyotrophic lateral sclerosis mutant. Biochemistry 39 (2000) 8125-32.
Livak, K.J.: Allelic discrimination using fluorogenic probes and the 5' nuclease assay. Genet Anal 14 (1999) 143-9.
Luo, D. and TA, R.: The regulation of catalase gene expression in mouse muscle cells is dependant on the CCAAT-binding factor NF-Y. Biochemical and Biophysical Research Communications 303 (2003) 609-618.
Lyrenäs, L., Wincent, E., Forsberg, L. and Morgenstern, R.: The Potential Impact of Polymorphism on Oxidative Stress Status. In: Cadenas, E. (Ed.), Redox-Genome Interactions in Health and Disease. Marcel Dekker, Inc., New York, 2004.
Lyrenäs, L., Zotova, E., Ekström, L. and Morgenstern, R.: Oxidative stress, genetic variation and disease. In: Quek, J. (Ed.). World Scientific Publishing Co., 2005.
Maiorino, M., Bosello, V., Ursini, F., Foresta, C., Garolla, A., Scapin, M., Sztajer, H. and Flohe, L.: Genetic variations of gpx-4 and male infertility in humans. Biol Reprod 68 (2003) 1134-41.
Maire, P., Wuarin, J. and Schibler, U.: The role of cis-acting promoter elements in tissue-specific albumin gene expression. Science 244 (1989) 343-6.
Maity, S.N. and de Crombrugghe, B.: Role of the CCAAT-binding protein CBF/NF-Y in transcription. Trends Biochem Sci 23 (1998) 174-8.
Maity, S.N., Golumbek, P.T., Karsenty, G. and de Crombrugghe, B.: Selective activation of transcription by a novel CCAAT binding factor. Science 241 (1988) 582-5.
Mancuso, M., Filosto, M., Naini, A., Rocchi, A., Del Corona, A., Sartucci, F., Siciliano, G. and Murri, L.: A screening for superoxide dismutase-1 D90A mutation in Italian patients with sporadic amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord 3 (2002) 215-8.
Mantovani, R.: A survey of 178 NF-Y binding CCAAT boxes. Nucleic Acids Res 26 (1998) 1135-43.
Mantovani, R.: The molecular biology of the CCAAT-binding factor NF-Y. Gene 239 (1999a) 15-27.
Mantovani, R.: The molecular biology of the CCAAT-binding factor NF-Y. Gene 239 (1999b) 15-27.
Marklund, S.L.: Extracellular superoxide dismutase in human tissues and human cell lines. J Clin Invest 74 (1984) 1398-403.
Marklund, S.L., Nilsson, P., Israelsson, K., Schampi, I., Peltonen, M. and Asplund, K.: Two variants of extracellular-superoxide dismutase: relationship to cardiovascular risk factors in an unselected middle-aged population. J Intern Med 242 (1997) 5-14.
Mattey, D.L., Hassell, A.B., Dawes, P.T., Jones, P.W., Yengi, L., Alldersea, J., Strange, R.C. and Fryer, A.A.: Influence of polymorphism in the manganese superoxide dismutase locus on disease outcome in rheumatoid arthritis: evidence for

interaction with glutathione S-transferase genes. Arthritis Rheum 43 (2000) 859-64.
Matuoka, K. and Chen, K.Y.: Transcriptional regulation of cellular ageing by the CCAAT box-binding factor CBF/NF-Y. Ageing Res Rev 1 (2002) 639-51.
Mironov, A.A., Fickett, J.W. and Gelfand, M.S.: Frequent alternative splicing of human genes. Genome Res 9 (1999) 1288-93.
Moscow JA, S.L., Ingram DT, Gnarra J, Johnson B, Cowan KH.: Loss of heterozygosity of the human cytosolic glutathione peroxidase I gene in lung cancer. Carcinogenesis. (12) (1994) 2769-73.
Mosialou, E., Ekstrom, G., Adang, A.E. and Morgenstern, R.: Evidence that rat liver microsomal glutathione transferase is responsible for glutathione-dependent protection against lipid peroxidation. Biochem Pharmacol 45 (1993) 1645-51.
Mosialou, E., Piemonte, F., Andersson, C., Vos, R.M., van Bladeren, P.J. and Morgenstern, R.: Microsomal glutathione transferase: lipid-derived substrates and lipid dependence. Arch Biochem Biophys 320 (1995) 210-6.
Nakashima, H., Yamamoto, M., Goto, K., Osumi, T., Hashimoto, T. and Endo, H.: Isolation and characterization of the rat catalase-encoding gene. Gene 79 (1989) 279-88.
Narlikar, G.J., Fan, H.Y. and Kingston, R.E.: Cooperation between complexes that regulate chromatin structure and transcription. Cell 108 (2002) 475-87.
Nenoi, M., Ichimura, S., Mita, K., Yukawa, O. and Cartwright, I.L.: Regulation of the catalase gene promoter by Sp1, CCAAT-recognizing factors, and a WT1/Egr-related factor in hydrogen peroxide-resistant HP100 cells. Cancer Res 61 (2001) 5885-94.
Nicholls, P., van Buuren, K.J. and van Gelder, B.F.: Biochemical and biophysical studies on cytochrome aa 3 . 8. Effect of cyanide on the catalytic activity. Biochim Biophys Acta 275 (1972) 279-87.
Ogata, M.: Acatalasemia. Hum Genet 86 (1991) 331-40.
Ogata, M., Mizugaki, J., Taketa, K. and Takahara, S.: Activities of catalase in leucocytes and glucose-6-phosphate dehydrogenase in erythrocytes of hypocatalasemia and acatalasemia. Tohoku J Exp Med 122 (1977a) 93-7.
Ogata, M., Mizugaki, J., Ueda, K. and Ikeda, M.: Activities of superoxide dismutase and glutathione peroxidase in the red cells of Japanese acatalasemia blood. Tohoku J Exp Med 123 (1977b) 95-8.
Ogata, M., Tomokuni, K., Watanabe, S., Osaki, H. and Sadamoto, M.: Residual catalase in the blood of Japanese acatalasemia. Tohoku J Exp Med 107 (1972) 105-14.
Orlando, V., Strutt, H. and Paro, R.: Analysis of chromatin structure by in vivo formaldehyde cross-linking. Methods 11 (1997) 205-14.
Orrell, R.W., Marklund, S.L. and deBelleroche, J.S.: Familial ALS is associated with mutations in all exons of SOD1: a novel mutation in exon 3 (Gly72Ser). J Neurol Sci 153 (1997) 46-9.
Parboosingh, J., Rouleau, G., Meninger, V., McKenna-Yasek, D., Brown Jr, R. and Figlewicz, D.: Abscence of mutations in the Mn superoxide dismutase or catalase genes in familial amyotrophic lateral sclerosis. Neuromusc. Disord. 5 (1995) 7-10.
Parhami, F., Fang, Z.T., Fogelman, A.M., Andalibi, A., Territo, M.C. and Berliner, J.A.: Minimally modified low density lipoprotein-induced inflammatory responses in endothelial cells are mediated by cyclic adenosine monophosphate. J Clin Invest 92 (1993) 471-8.
Parton, M.J., Broom, W., Andersen, P.M., Al-Chalabi, A., Nigel Leigh, P., Powell, J.F. and Shaw, C.E.: D90A-SOD1 mediated amyotrophic lateral sclerosis: a single founder for all cases with evidence for a Cis-acting disease modifier in the recessive haplotype. Hum Mutat 20 (2002) 473.
Penrose, J.F., Spector, J., Baldasaro, M., Xu, K., Boyce, J., Arm, J.P., Austen, K.F. and Lam, B.K.: Molecular cloning of the gene for human leukotriene C4 synthase. Organization, nucleotide sequence, and chromosomal localization to 5q35. J Biol Chem 271 (1996) 11356-61.
Percy, M.E.: Catalase: an old enzyme with a new role? Can J Biochem Cell Biol 62 (1984) 1006-14.

Pushpa-Rekha, T.R., Burdsall, A.L., Oleksa, L.M., Chisolm, G.M. and Driscoll, D.M.: Rat phospholipid-hydroperoxide glutathione peroxidase. cDNA cloning and identification of multiple transcription and translation start sites. J Biol Chem 270 (1995) 26993-9.
Qin, K. and Rosenfield, R.L.: Characterization of the basal promoter element of the human type 5 17beta-hydroxysteroid dehydrogenase gene. Biochim Biophys Acta 1728 (2005) 115-25.
Quan, F., Korneluk, R., Tropak, M. and Gravel, R.: Isolation and characterization of the human catalse gene. Nucleic Acids Res 14 (1986) 5321-5335.
Radi, R., Turrens, J.F., Chang, L.Y., Bush, K.M., Crapo, J.D. and Freeman, B.A.: Detection of catalase in rat heart mitochondria. J Biol Chem 266 (1991) 22028-34.
Rahman, S.H., Nanny, C., Ibrahim, K., O'Reilly, D., Larvin, M., Kingsnorth, A.J. and McMahon, M.J.: Genetic polymorphisms of GSTT1, GSTM1, GSTP1, MnSOD, and catalase in nonhereditary chronic pancreatitis: evidence of xenobiotic stress and impaired antioxidant capacity. Dig Dis Sci 50 (2005) 1376-83.
Ratnasinghe D, T.J., Andersen MR, Barrett MJ, Virtamo J, Taylor PR, Albanes D.: Glutathione peroxidase codon 198 polymorphism variant increases lung cancer risk. Cancer Res. 60(22) (2000) 6381-3.
Reimer, D.L., Bailley, J. and Singh, S.M.: Complete cDNA and 5' genomic sequences and multilevel regulation of the mouse catalase gene. Genomics 21 (1994) 325-36.
Reith, W., Siegrist, C.A., Durand, B., Barras, E. and Mach, B.: Function of major histocompatibility complex class II promoters requires cooperative binding between factors RFX and NF-Y. Proc Natl Acad Sci U S A 91 (1994) 554-8.
Reuterwall, C., Hallqvist, J., Ahlbom, A., de Faire, U., Diderrichsen, F., Högstedt, C., Pershagen, G., Theorell, T., Wiman, B. and Wolk, A.: Higher relative, but lower absolute risks of myocardial infarction in women than men: analysis of some major risk factors in the SHEEP study. Journal of Internal medicine 246 (1999) 161-174.
Rosen, D.R., Siddique, T., Patterson, D., Figlewicz, D.A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'Regan, J.P., Deng, H.X. and et al.: Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362 (1993) 59-62.
Sampson, A.P., Siddiqui, S., Buchanan, D., Howarth, P.H., Holgate, S.T., Holloway, J.W. and Sayers, I.: Variant LTC(4) synthase allele modifies cysteinyl leukotriene synthesis in eosinophils and predicts clinical response to zafirlukast. Thorax 55 Suppl 2 (2000) S28-31.
Sanak, M., Simon, H.U. and Szczeklik, A.: Leukotriene C4 synthase promoter polymorphism and risk of aspirin-induced asthma. Lancet 350 (1997) 1599-600.
Sanak, M. and Szczeklik, A.: Genetics of aspirin induced asthma. Thorax 55 Suppl 2 (2000) S45-7.
Sandstrom, J., Nilsson, P., Karlsson, K. and Marklund, S.L.: 10-fold increase in human plasma extracellular superoxide dismutase content caused by a mutation in heparin-binding domain. J Biol Chem 269 (1994) 19163-6.
Sayers, I., Barton, S., Rorke, S., Sawyer, J., Peng, Q., Beghe, B., Ye, S., Keith, T., Clough, J.B., Holloway, J.W., Sampson, A.P. and Holgate, S.T.: Promoter polymorphism in the 5-lipoxygenase (ALOX5) and 5-lipoxygenase-activating protein (ALOX5AP) genes and asthma susceptibility in a Caucasian population. Clin Exp Allergy 33 (2003) 1103-10.
Segovia-Silvestre, T., Andreu, A.L., Vives-Bauza, C., Garcia-Arumi, E., Cervera, C. and Gamez, J.: A novel exon 3 mutation (D76V) in the SOD1 gene associated with slowly progressive ALS. Amyotroph Lateral Scler Other Motor Neuron Disord 3 (2002) 69-74.
Sen, C.K. and Packer, L.: Antioxidant and redox regulation of gene transcription. Faseb J 10 (1996) 709-20.
Shimoda-Matsubayashi, S., Matsumine, H., Kobayashi, T., Nakagawa-Hattori, Y., Shimizu, Y. and Mizuno, Y.: Structural Dimorphism in the Mitochondrial

Targeting Sequence in the Human Manganese Superoxide Dismutase Gene. Biochem Biophys Res Commun 226 (1996) 561-5.
Sinha, S., Maity, S.N., Lu, J. and de Crombrugghe, B.: Recombinant rat CBF-C, the third subunit of CBF/NFY, allows formation of a protein-DNA complex with CBF-A and CBF-B and with yeast HAP2 and HAP3. Proc Natl Acad Sci U S A 92 (1995) 1624-8.
Stathopulos, P.B., Rumfeldt, J.A., Scholz, G.A., Irani, R.A., Frey, H.E., Hallewell, R.A., Lepock, J.R. and Meiering, E.M.: Cu/Zn superoxide dismutase mutants associated with amyotrophic lateral sclerosis show enhanced formation of aggregates in vitro. Proc Natl Acad Sci U S A 100 (2003) 7021-6.
Strachan, T. and Read, A.: Human Molecular genetics 2. John Wiley & Sons, Inc., New York, 1999.
Sueyoshi, T. and Negishi, M.: Phenobarbital response elements of cytochrome P450 genes and nuclear receptors. Annu Rev Pharmacol Toxicol 41 (2001) 123-43.
Suske, G.: The Sp-family of transcription factors. Gene 238 (1999) 291-300.
Suzuki, Y.J., Mizuno, M. and Packer, L.: Transient overexpression of catalase does not inhibit TNF- or PMA-induced NF-kappa B activation. Biochem Biophys Res Commun 210 (1995) 537-41.
Szelestei, T., Bahring, S., Wagner, Z., Aydin, A., Molnar, G.A., Kocsis, B., Nagy, J. and Wittmann, I.: Serum levels of L-arginine analogues and glutathione peroxidase and catalase gene variants in Type 2 diabetes mellitus patients. Diabet Med 22 (2005) 356-7.
Thameem, F., Yang, X., Permana, P.A., Wolford, J.K., Bogardus, C. and Prochazka, M.: Evaluation of the microsomal glutathione S-transferase 3 (MGST3) locus on 1q23 as a Type 2 diabetes susceptibility gene in Pima Indians. Hum Genet 113 (2003) 353-8.
Tomblyn, M., Kasarskis, E.J., Xu, Y. and St Clair, D.K.: Distribution of MnSOD polymorphisms in sporadic ALS patients. J Mol Neurosci 10 (1998) 65-6.
Tomkins, J., Banner, S.J., McDermott, C.J. and Shaw, P.J.: Mutation screening of manganese superoxide dismutase in amyotrophic lateral sclerosis. Neuroreport 12 (2001) 2319-22.
Van Landeghem, G.F., Tabatabaie, P., Beckman, G., Beckman, L. and Andersen, P.M.: Manganese-containing superoxide dismutase signal sequence polymorphism associated with sporadic motor neuron disease. Eur J Neurol 6 (1999) 639-44.
Van Sambeek, R., Stevenson, D.D., Baldasaro, M., Lam, B.K., Zhao, J., Yoshida, S., Yandora, C., Drazen, J.M. and Penrose, J.F.: 5' flanking region polymorphism of the gene encoding leukotriene C4 synthase does not correlate with the aspirin-intolerant asthma phenotype in the United States. J Allergy Clin Immunol 106 (2000) 72-6.
Welsch, D.J., Creely, D.P., Hauser, S.D., Mathis, K.J., Krivi, G.G. and Isakson, P.C.: Molecular cloning and expression of human leukotriene-C4 synthase. Proc Natl Acad Sci U S A 91 (1994) 9745-9.
Wen, J.K., Osumi, T., Hashimoto, T. and Ogata, M.: Molecular analysis of human acatalasemia. Identification of a splicing mutation. J Mol Biol 211 (1990) 383-93.
Winter JP, G.Y., Grant PJ, Wild CP.: Glutathione peroxidase 1 genotype is associated with an increased risk of coronary artery disease. Coron Artery Dis. Apr;14(2) (2003) 149-53.
Wright, K.L., Moore, T.L., Vilen, B.J., Brown, A.M. and Ting, J.P.: Major histocompatibility complex class II-associated invariant chain gene expression is up-regulated by cooperative interactions of Sp1 and NF-Y. J Biol Chem 270 (1995) 20978-86.
Yamada, H., Yamada, Y., Adachi, T., Goto, H., Ogasawara, N., Futenma, A., Kitano, M., Hirano, K. and Kato, K.: Molecular analysis of extracellular-superoxide dismutase gene associated with high level in serum. Jpn J Hum Genet 40 (1995) 177-84.
Yoshimura, S., Suemizu, H., Taniguchi, Y., Arimori, K., Kawabe, N. and Moriuchi, T.: The human plasma glutathione peroxidase-encoding gene: organization, sequence and localization to chromosome 5q32. Gene 145 (1994) 293-7.

Zhu, Z., Mukhina, S., Zhu, T., Mertani, H.C., Lee, K.O. and Lobie, P.E.: p44/42 MAP kinase-dependent regulation of catalase by autocrine human growth hormone protects human mammary carcinoma cells from oxidative stress-induced apoptosis. Oncogene 24 (2005) 3774-85.

Zotova, E., Savost´yanov, K., Christyakov, D., Bursa, T., Galeev, I., Strokov, I. and Nosikov, V.: Association of Polymorphic Markers of the Antioxidant Enzyme Genes with Diabetic Polyneuropathy in Type 1 Diabetes Mellitus. Molecular Biology(Molekulyarnaya Biologiya) 38 (2004) 244-249.

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