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master in de biomedische wetenschappen: milieu en gezondheid

Masterproef

Interindividual Differences in Response to Blueberry Juice Intervention in Healthy Human Subjects: A Genomics Approach

Promotor :
dr. S. VAN BREDA

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Masterproef voorgedragen tot het bekomen van de graad van master in de biomedische wetenschappen , afstudeerrichting milieu en gezondheid

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Interindividual Differences in Response to Blueberry Juice Intervention in Healthy Human Subjects: A Genomics Approach

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Abstract

Background: A variety of dietary components can alter gene expression and at the same time the genetic makeup of an individual may coordinate its response to diet. Antioxidants, such as blueberry, are known to have protective effects against age related diseases but studies that examine their mechanism of action in human are lacking.

Objective: This study aims to investigate the possible interactions between blueberry-apple juice and single-nucleotide polymorphisms (SNPs) in drug metabolizing and DNA repair genes, in particular GSTT1 and XRCC1, in order to identify molecular pathways associated with polymorphisms which may be contributing to health benefits of blueberry intervention.

Design: The present study is follow-up to a previous study to assess the impact of genetic polymorphisms following a dietary blueberry intervention and investigates transcriptomic changes. This study will investigate the whole genome gene expression changes in lymphocytes of healthy human subjects receiving a four-week blueberry-apple juice intervention in pre- identified subgroups based on their outcome of intervention on protection against oxidative DNA damage. To our knowledge, this is the first research of whole genome gene expression analysis together with the impact of genetic polymorphism following a dietary intervention in humans.

Subjects and Methods: The study population consists of 147 healthy subjects, including 46 male and 101 female subjects with average age of 28 ± 1 year, who met the inclusion criteria and for whom transcriptomic data is available. In this study, the impact of genetic variations of the GSTT1*0 (wildtype and deletion) and XRCC1*4 (wildtype, heterozygote and homozygote) following a 4-week blueberry intervention is reviewed.
Two-Colour Microarray-Based Gene Expression Analysis is used to find significantly modulated genes (pair wise t-tests, $p < 0.05$). Significantly correlated genes ($p < 0.05$) are analyzed in MetaCore™ to identify involvement in specific biological pathways. Gene networks based on modulated genes are created in MetaCore™ using the shortest paths algorithm.
Results: MetaCore GeneGo analysis of cellular processes for subjects following the 4-week blueberry intervention, identified top important pathways that are significantly ($p < 0.05$) modulated in the GSTT1*0 and XRCC1*4 variants.
Regarding GSTT1*0 wildtype variant, the significant pathways found are predominantly part of the immune response while for the GSTT1*0 deletion variants, significant pathways are related to G-protein signalling. Regarding the XRCC1*4 wildtype variant, the significant pathways modulated are found to be mainly related to immune response while for the XRCC1*4 heterozygous and homozygous, significantly modulated pathways are mainly related to glutathione metabolism.
Conclusion: This study provides insight into the relevant molecular targets on gene expression levels modulated after a blueberry intervention. These data illustrate that chemoprotective actions of berry phytochemicals on defence against DNA damage and degenerative diseases are related to certain signalling pathways which are of biological relevance in the etiology of chronic diseases. More studies in humans are needed to elucidate the relevance and magnitude of nutrient-gene interactions.
Key words: *Blueberry, GSTT1*1, XRCC1*4, Polymorphism, DNA damage, Oxidative stress*

Introduction

In order to survive, we spend a lot of money and energy making food choices every day that impact our health. It is increasingly appreciated that the chances of developing chronic diseases such as diabetes, cardiovascular disease and cancer are significantly affected by the choice of our lifestyle. Intake of fruits and vegetables is thought to protect against the development of many cancers. Healthy lifestyle behaviours for cancer risk reduction include a healthy diet. Several studies have shown that chemopreventive agents possess strong cancer-preventive properties that might interrupt different stages of cancer. Epidemiological studies suggest that cancer risk can be reduced by an overall dietary pattern that favours a high intake of plant foods rich in a wide range of phytochemicals (1, 2, 3, and 4).

To prevent, the onset of cancer, the National Institutes of Health (NIH) recommends a high fibre, low fat diet, consisting of more fruits and vegetables. In recent years, there has been increasing interest in the use of dietary factors rich in chemopreventive substances and several studies indicate that consumption of fruits and vegetables are a suggested way of defence against DNA damage and degenerative diseases (2, 4). Several natural products, such as, grains, nuts, cereals, spices, fruits, vegetables, and herbs and their various phytochemical constituents including, phenolics, flavonoids, carotenoids, alkaloids, nitrogen containing as well as organosulfur compounds confer protective effects against wide range of cancers(5).

Oxygen is essential for metabolic processes in the body. Oxidative stress is the condition where the balance between oxidising and reducing compounds is disrupted. Oxygen derived free radicals known as reactive oxygen species (ROS) are reported to exert detrimental effects, such as membrane lipid peroxidation, alteration of lipid-protein interactions, enzyme inactivation, and DNA breakage. Excess in radicals, may interact with biomaterials and thus induce cellular oxidative damage. ROS such as H_2O_2 and hydroxyl radicals readily damage biological molecules including DNA and protein which can eventually lead to apoptotic or necrotic cell death. Therefore, removal of excess ROS or suppression of their generation by antioxidants may be effective in preventing oxidative cell death (6).

There are increasing evidences that antioxidants such as polyphenols may protect cell constituents against oxidative damage and, therefore, limit the risk of various degenerative diseases associated with oxidative stress. Research on the effects of berry intake on human cardiovascular and neurological diseases, diabetes and cancer has increased significantly in the past 10–15 years. Scavenging of reactive oxygen species and reactive carcinogen metabolites, as well as induction of phase II detoxification enzymes may explain the suggested anticarcinogenic potential of fruits and vegetables (7 and 8).

Blueberries are perennial flowering plants with indigo-coloured berries in the section Cyanococcus within the genus Vaccinium. The flowers are bell-shaped, white, pale pink or red, sometimes tinged greenish. The fruit is a berry with a flared crown at the end; they are pale greenish at first, then reddish-purple, and finally dark blue when ripe. They have a sweet taste when mature, with variable acidity. Blueberries are ranked very highly among fruits and vegetables for their antioxidant capacity. A higher antioxidant capacity has been

reported in blueberry that can neutralize free radicals which cause neurodegenerative disease, cardiovascular disease, and cancer. Polyphenols are naturally occurring compounds found largely in the fruits, vegetables, cereals and beverages. Berries contains up to 200–300 mg polyphenols per 100 grams fresh weight. Polyphenols are secondary metabolites of plants and are generally involved in defence against ultraviolet radiation or aggression by pathogens. Polyphenols and other food phenolics are the subject of increasing scientific interest because of their possible beneficial effects on human health (8).

Flavonoids comprise the most studied group of polyphenols. This group has a common basic structure consisting of two aromatic rings bound together by three carbon atoms that form an oxygenated heterocycle. Flavonoids comprise about 4000 different structures, varying in occurrence in the food chain and in chemical properties. Flavonoids can be subdivided into some of the following classes: flavanols, flavanones, flavonols, flavones and anthocyanins (9). Flavonoids form a class of naturally occurring polyphenolic compounds, many of which provide attractive colours to flowers, fruit and leaves. Flavonoids are found in high quantities in blueberries and are claimed to be able to protect against certain forms of cancer and aging, possibly by preventing initial DNA damage. Flavonoids comprise about 4000 different structures, varying in occurrence in the food chain and in chemical properties. Flavonoids can be subdivided into some of the following classes: flavanols, flavanones, flavonols, flavones and anthocyanins (10). Quercetin, myricetin, catechins etc., are some most common flavonoids.

Quercetin, the best studied flavonoid, is present in high levels in blueberries. Quercetin, being a strong anti-oxidant, is renowned as scavengers for highly reactive species and has been shown to protect against oxidative DNA damage in vitro in human lymphocytes (11).

Blueberry also contains other flavonoids such as anthocyanins and catechins which also possess antioxidant capacity and these components may also attribute to the anticarcinogenic properties as observed. Anthocyanins present in berries provide the natural pigmentation and exhibit a wide range of antioxidant protection and therapeutic benefits including the integrity of genomic DNA, anti-inflammatory, and anticarcinogenic properties (12). Anthocyanins are novel antioxidants and potent inhibitors of lipid peroxidation which trigger genetic signalling in promoting human health and disease prevention (13).

Epidemiological studies have repeatedly shown an inverse association between the risk of chronic diseases and the consumption of polyphenolic rich diet. The phenolic groups in polyphenols can accept an electron to form relatively stable phenoxyl radicals, thereby disrupting chain oxidation reactions in cellular components. Many studies have been performed on individual phytochemicals found in blueberries in relation to their chemopreventive activity (14, 15, 16), but only a few have investigated the effect of a blueberry dietary intervention on biomarkers of disease risk in humans.

Evidence that genetic variation at genes involved in the etiology of chronic diseases could interact with environmental exposures, such as diet, to modulate individuals' susceptibility to developing these conditions support the notion that more tailored dietary recommendations may be helpful in the prevention of disease related co morbidities.
Epigenetic regulation is a modification of DNA without a change in the sequence that results in a change in gene expression or phenotype. Unlike mutations in the genetic code, these epigenetic alterations may be modifiable. Coordination of histone acetylation and

deacetylation is an important regulatory mechanism for gene expression. In cancer, the balance between acetylation and deacetylation is often deregulated, and tumour suppressor genes are frequently silenced. Constituents in the diet, including berries, may have the potential to alter a number of these epigenetic events (64 and 65).

In order to explore the mechanism underlying the beneficial health effects of blueberries, Wilms et al. investigated a direct association between increased flavonoid intake in healthy volunteers and protection at the biomarker level. Furthermore, in a pilot study on supplementation of healthy volunteers by administering a quercetin-rich blueberry/apple juice, Wilms demonstrated that a 4-week intervention period is suitable for enhancing antioxidant defence, and that a quercetin plasma level was reached that reduced ex vivo induced DNA damage (17).

In a more recent study, Wilms explored the impact of multiple genetic polymorphisms on effects of a 4-week blueberry juice intervention on ex vivo induced lymphocytic DNA damage in 168 healthy human volunteers. The researchers reported that subjects bearing specific genetic polymorphisms in drug metabolizing and DNA repair genes, in particular GSTT1- and XRCC1 wild-type variants may benefit more from DNA damage-protecting effects of berries than their genetic counterparts (18).

Glutathione S-transferase theta 1 (GSTT1) is a member of a super family of proteins, mainly known as phase II enzymes. Drugs, poisons, and other compounds are usually modified by the phase I and/or phase II mechanisms, and finally excreted from the body. GSTs contribute to this type of metabolism by conjugating these compounds with reduced glutathione to facilitate dissolution in the aqueous cellular and extracellular media, and, from there, out of the body. They also play an important role in protection against oxidative stress (22).

Endogenous and environmental agents can cause DNA damage in cells. Variability in DNA repair genes may contribute to human cancer risk. Dietary mutagenic chemicals, ultraviolet and ionizing radiation, and heavy metals are environmental agents that damage the genome, causing DNA cross-links, adducts, and oxidative cleavage. DNA single-strand breaks can be induced by endogenous reactive molecules such as reactive oxygen species and pose a continuous threat to genetic integrity. X-ray cross-complementing group 1 (XRCC1) protein plays a major role in facilitating the repair of single-strand breaks via an ability to interact with multiple enzymatic components of repair reactions (23).

Several studies have reported that polymorphisms in GSTT1 and/ or XRCC1 can alter the rate of apoptosis and intracellular reactive oxygen species (ROS) levels (19, 20, 22, and 23). Thus, evaluation of the role of genetic polymorphisms in polymorphisms in the above mentioned drug metabolizing and DNA repair genes may provide a helpful tool in assessing susceptible groups that benefit from specific dietary interventions.

Moreover, despite the fact that our understanding of some of the potential mechanisms of berry phytochemicals and their antioxidant, anti- inflammatory and anti-angiogenesis effects has increased over the past decade, studies in humans are are still needed to better illustrate the cause-and-effect relationship between blueberry antioxidant intake and their chemo-preventive actions in defence against DNA damage and degenerative diseases. At present, the genetic pathways associated with the genetic polymorphisms that contribute to

health benefits of berries are not yet known. The wide consumption of berry fruits and their potential impact on human health, demand an evaluation of the underlying mechanism. The current study will expand the understanding of the effects of blueberries on differential gene expression and the subsequent biological pathways in which they play a role. Through this approach, we hope to be able to elucidate the associations between blueberry intake and the (reduced) incidence of oxidative stress and cancer. For that reason, we will assess the contributions of genetic variations of GSTT1*0 and XRCC1*4 on biological pathways and cellular processes on outcomes of oxidative stress and cancer.

Furthermore, the evidence for the potential protective role of blueberries will be reviewed. In this unique unparalleled study, a comparison will be made between the cellular processes affected by berry intervention in different polymorphism of GSTT1 and XRCC1. It is essential to mention that, to our knowledge, this is the first investigation of whole genome gene expression analysis together with the impact of genetic polymorphism, following a dietary intervention in humans.

Using ArrayTrack™, lists of significantly differentially expressed genes following the 4-week blueberry intervention, were generated by means of pair wise t-tests ($p < 0.05$) in order to analyse the whole genome gene expression changes induced in lymphocytes before and after the blueberry intervention. Venn diagrams were created in which the significantly differentially expressed gene lists were incorporated for subjects showing the gene expressions occurring in the different polymorphisms of GSTT1*0 and XRCC1*4 separately. Next, the gene sets were exported from the Venn diagrams and transferred into MetaCore in order to identify the involvement of the genes in specific cellular GeneGo pathways.

Understanding the role of dietary interventions and their molecular mechanisms are essential in designing more personalized dietary interventions and have the potential to lead to therapies for broad-spectrum prevention and treatment of chronic diseases such as cardiovascular disease and cancer. Dietary approaches may hold promise as effective and safe preventive interventions for future.

Materials and Methods

Study Population

In the present study, we use the data characteristics of the subjects who had participated in a previous study by Wilms et al. (18) on the evaluation of a four-week blueberry/apple juice intervention, providing 97 mg quercetin and 16 mg ascorbic acid a day.
Genetic polymorphisms of genes involved in biotransformation, DNA repair and oxidative stress were expected to influence the antioxidative and antigenotoxic efficacy of intervention by micronutrients. Study subjects had a five day washout period for which they had to avoid dietary substances rich in flavonoids and quercetin.

Wilms study reported an increase in the total plasma antioxidant capacity (TEAC) and the defense against *ex vivo* induced oxidative DNA damage in lymphocytes (measured by comet assay) following a four-week blueberry intervention. Analysis of 34 genetic single nucleotide polymorphisms (SNP) in genes related to metabolism, oxidative stress, and DNA repair had shown that 6 SNPs significantly influenced the effect of the intervention, indicating that particular subpopulations, identified by specific gene-polymorphisms, may benefit more from the chemopreventive actions of blueberry than others.

Wilms study details a significant impact by GSTT1 polymorphism on the effect of blueberry juice intervention on plasma TEAC. More particularly, study reported that while the GSTT1*0 wildtypes may have the largest benefit from antioxidant intervention with regard to protection against ex- vivo induced oxidative DNA damage in lymphocytes, the GSTT1 deletion variants are the ones which show the largest increase in plasma antioxidative capacity (TEAC). It is of interest that GSTT1*0 deletion variants, are more susceptible to oxidative damage than the GSTT1* 0 wildtypes.

Additionally, Wilms study reported that lymphocytes of subjects carrying XRCC1*4 wildtype, showed a larger intervention related decrease in single strand breaks than lymphocytes from those carrying the XRCC1*4 heterozygous and homozygous variants.

Therefore a profound investigation of the modifications of gene expression level following the blueberry intervention is warranted. Investigating markers of oxidative stress and linking them to gene expression modulation at the whole genome level is an interesting approach, by which, we may be able to increase the understanding of molecular pathways accompanying changes in phenotypic biomarkers of disease risk. In this study, we will focus on GSTT1*0 and XRCC1*4 polymorphism in order to investigate different biological pathways and cellular processes in response to antioxidants received through the dietary intervention.

Microarray Data Analysis

In the whole study population, subgroups were defined based on the significant outcome of the intervention on protection against DNA damage as measured by the comet assay. and subgroups were created according to polymorphism. This approach enables the identification of the relevant pathways and biological processes which are indicative for the chemopreventive action of a fruit-juice rich in phytochemicals.

As the whole study population is genetically heterogenic, subjects were genotyped for 34 single nucleotide polymorphisms associated with oxidative stress; biotransformation and DNA repair (Table 1). With respect to the effect on oxidative DNA damage, the polymorphisms GSTT1*0 and XRCC1*4 were significant predictors.

Using ArrayTrack™, for each group, gene lists comprising genes which were significantly differentially expressed after the intervention were generated by means of pair wise t-tests ($p < 0.05$) in which each subjects acted as its own control. The Benjamini–Hochberg false discovery rate (FDR) at this p-value was <15%.
To correlate gene expression data for the different subgroups with the measured difference in mean tail moment as parameter of DNA damage, Spearman's rank correlation analyses were used. Only genes present in at least 70% of subjects were used. Prior to correlation analysis, missing values were imputed by finding the k nearest neighbors (k was set to 15), using a Euclidean metric and imputing the missing elements by averaging the (non-missing) elements of its neighbors. The online Gene Expression Profile Analysis Suite (GEPAS, http://gepas.bioinfo.cipf.es/) was used to perform the Spearman's correlation analyses.

Next, Venn diagrams were created in ArrayTrack™ in which the significantly differentially expressed genes were incorporated for each group, thereby showing the number of genes which were specifically modulated in each subgroup separately (Table 2).

To identify the involvement of the genes in specific cellular GeneGo pathways, the gene sets were transferred into the online commercially software suite MetaCore™ version 6.1 (GeneGo, San Diego, CA). Pathways with a p-value < 0.05 were considered significantly modulated.
Gene networks based on a select number of genes for a selection of cellular processes were created in MetaCore™ using "Dijkstra's shortest path" algorithm. The resulting networks were prefiltered on a sub-cellular level indicating the localization of gene expression. By default, these sub-cellular levels comprise of extracellular, membrane, cytoplasm and nucleus. From each network, those genes of which the gene product eventually result in a cellular effect were defined as target genes and grouped at the baseline of the network. Based on the interpretation of gene annotation and function retrieved from EntrezGene (http://www.ncbi.nlm.nih.gov/gene/) and MetaCore™, the modulated target genes were linked to the involved biological processes.

Results

In the current study, in order to identify the pathways and processes significantly modulated in lymphocytes of Wilms study subjects, we utilized the significant gene lists of GSTT1*0 and XRCC1*4 and examined their involvement in Metacore^{TM} GeneGo cellular processes and biological pathways (Tables 3, 4, 5 and 6).

The top twenty pathways and cellular processes significantly associated with the difference in ex-vivo induced oxidative DNA damage in lymphocytes of subjects, as listed in Tables 5 and 6, are further visualized in Venn diagrams, for the results of correlation analysis of GSTT1*0 and XRCC1*4 respectively (Figures 1 and 2).

Comparison of the results between the paired t-test and correlation analysis showed that biological pathways impacted are mostly different. Analysis shows that consumption of a diet rich in blueberry antioxidants in the GSTT1*0 and XRCC1*4 wildtype variants trigger the response through the immune system. While in the GSTT1*0 null genotype subjects, the blueberry intervention triggers the G-protein signalling pathways. In the case of XRCC1*4 heterozygote and homozygote variants, impacts on glutathione metabolism pathways are observed. Figures 10 and 11 represent a visual and comprehensive summary of results of this investigation in GSTT1*0 and XRCC1*4 variants, respectively.

A more detailed biological interpretation of the data will focus on the identified pathways in each subgroup, namely, the immune response in GSTT1*0 wt and XRCC1*4 wt variants and the G-protein signalling in the GSTT1*0 deletion variant. In addition, we will discuss a selection of significant gene modulations (up- or down- regulation) observed in these processes following the blueberry intervention. Since the gene expression changes in XRCC1*4 heterozygote and homozygote variants are not as significant when compared to the wildtypes, we will only briefly review the glutathione metabolism pathway in this group.GeneGo analysis of cellular processes in the networks related to significant biological pathways in each variant subgroup will be presented in this study.

GSTT1*0 Variants

Using GeneGo by Metacore^{TM}, several biological pathways were found to be significantly ($p < 0.05$) modulated in both the GSTT1*0 wildtype and the deletion variants. The results at the level of pathways were visualized using Venn diagram of GeneGo biological pathways. Overall comparison of the results between the paired t-test and correlation analyses of significantly modulated pathways for GSTT1*0 deletion and GSTT1*0 wt variants showed no similarity at the level of specific biological pathways (Figure 1).

As for the GSTT1*0 wild types, most of the gene expression changes occurred in biological pathways involved in the immune response while for GSTT1*0 deletion variants significant pathways for the correlating genes were found to be part of the cellular processes G-protein signaling (Table 6).

Networks of modulated genes were identified for both variants using GeneGo shortest path networks. As for the GSTT1*0 wildtypes, significant networks include regulation of cell death and apoptosis, lipid catabolic process, phospholipid and organophosphate metabolic processes and regulation of platelet activation. Whereas for the GSTT1*0 deletion variants, significant networks of modulated genes include intracellular signal transduction, wound healing, platelet activation, regulation of body fluid, response to external and endogenous stimuli and to peptide hormones and nerve growth factor receptor signaling (Table 8).

In the following pages we will review and discuss the cellular processes, significant gene modulations and gene networks for each of the subgroups under investigation.

I. GSTT1*0 Wildtypes

Gene expression changes following the 4-week blueberry intervention in lymphocytes of subjects bearing GSTT1*0 wt mostly involved immune response pathways. Biological functions of these cellular processes are diverse and among the highest ranking by GeneGo Metacore™ (Table 6).

The top 5 immune response pathways that were significantly modulated in GSTT1*0 wt variants include:

a. HGMB1 release from the cell
b. HMGB/TLR signaling
c. CD16 signaling in NK cells
d. PGE2 in immune and neuroendocrine system interactions
e. IFN gamma signaling

In the immune response cellular processes listed above, several genes were modulated. Those involved in significant networks of cellular processes identified by GeneGo will be summarized below. Gene networks for the immune response biological processes were generated in MetaCore™ in order to visualize the complex set of connections between the genes and their interactions (Figures 3 and 4).
In this section, we will review the immune response pathways and provide relevant details on modulated genes and related networks.

a. Immune response- High mobility group box 1 (HMGB1) release from the cell and
b. Immune response- HMGB/TLR signaling

HMGB1, an evolutionarily conserved chromosomal protein, was recently re-discovered to act as a “danger signal” (alarmin) to alert the innate immune system for the initiation of host defense or tissue repair (24). HMGB1 is a nuclear transcription factor. It is the prototypic damage-associated molecular pattern molecule that has been implicated in several inflammatory disorders. HMGB1 is the multifunctional protein. Primarily, it is the nuclear DNA-binding protein. Being released into the extracellular milieu either by active secretion by cell or by passive release from damaged or necrotic cells, HMGB1 can function as a cytokine.

HMGB1 exerts different biological functions dependent on its cellular localization. Once released from cells, HMGB1 can mediate inflammatory response. Once HMGB1 is released by damaged cells or activated immune cells, it acts as danger molecule and triggers the inflammatory signaling cascade. Currently, evidence is accumulating that post-translational modifications such as oxidation may modulate the pro-inflammatory potential of danger signals (25).

c. Immune response- CD 16 signaling in NK cells:

Besides the HGMB1 release and signaling pathways, members of the CD16 signaling pathway were also modulated**.** Accumulating data has highlighted the importance of NK cells in host immune response against cancer and in therapy-induced antitumor response. The recognition and the lyses of tumor cells by NK cells are regulated by a complex balance of inhibitory and activating signals. The majority of NK cells in blood or at inflammation sites have a a strong expression of CD16. These cells possess a high cytotoxic potential NK cells have a role in immuno surveillance of cancer and the ability to prevent the tumor growth (26, 27, and 28).

d. Immune response- PGE2 in immune and neuro endocrine systems interactions

Besides the HGMB1 release and signaling and CD16 signaling pathways, members of PGE2 neuroendocrine system were also modulated. During systemic infections, the immune system can generate signals in the brain and act on different neuronal circuits via soluble molecules such as pro-inflammatory cytokines that act on the cells forming the blood-brain barrier and the circumventricular organs. These activated cells release Prostaglandin E2 (PGE2) that triggers pathways involved in the control of homeostasis and provide inhibitory feedback to the innate immune system. Activation of circuits that control plasma release of Glucocorticoids is probably the most critical among such neurophysiologic functions for the survival of the host affected by pathogens (29).

e. **Immune Response- Interferon-gamma (IFN gamma) signaling**

Besides the HGMB1 release and signaling , CD16 signaling and the PGE2 neuroendocrine system pathways, members of the IFN gamma signaling pathways were also modulated. Interferons (IFNs) are pleiotropic cytokines that mediate anti-viral responses, inhibit proliferation and participate in immune surveillance and tumor suppression by inducing the transcription of a number of IFN-stimulated genes. Interferon-gamma (IFN-gamma) is produced by activated T cells and natural killer (NK) cells (22).

There are many known STAT1-targets in IFN-gamma-mediated signaling. These are SMAD family member 7 (SMAD7), Interferon regulatory factor 1 (IRF1) and proteins involved in cell cycle regulation. Several proteins interact with STAT1 and modulate its transcriptional activity: CREB-binding proteins (CBP and p300), Minichromosome maintenance protein 5 (MCM5) and Breast cancer susceptibility gene 1 (BRCA1) (23). In addition, IFN-gamma may activate JAK-STAT-independent pathways.

In the immune response cellular processes, impacted by the 4-week blueberry dietary intervention, several genes were modulated (Table 7) for which we will include a brief summary in the following section.

Overall, an up regulation of CREB binding protein (CBP), Cyclin-dependent kinase inhibitor 1A (p21, Cip1), nucleolar protein 3 (NOL3), TNF receptor-associated factor 6 (TRAF6), apoptosis-related cysteine peptidase (Caspase 3), Calmodulin (CALM1) have and a down regulation of Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1(NF-kB), (TNFRSF6)-associated via death domain (FADD), TNF receptor super family, member 6 (FAS R), Phospholipase C and gamma 1 (PLC gamma) have been observed in this study.

Furthermore, in order to visualize the complex set of connections between the genes and their interactions two gene networks for the immune response pathways were generated in MetaCore™ as identified by GeneGo analysis using shortest paths networks. The network of genes including p21, CBP, TRAF6, RelB (NF-*k*B subunit) and NOL3 are involved in regulation of cell death and apoptosis. The network of genes including Calmodulin, Caspase-3, FasR(CD95), PLC-gamma 1 and FADD are involved in lipid catabolic process, phospholipid metabolic process, organophosphate metabolic process, positive regulation of molecular function and platelet activation (Figures 3 and 4).

Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1(NF-*k*B):

NF-*k*B is a transcription regulator that is activated by various intra- and extra-cellular stimuli and has been detected in numerous cell types that express cytokines, chemokines, growth factors, cell adhesion molecules, and some acute phase proteins in health and in various disease states.

NF-*k*B is activated by a wide variety of stimuli such as cytokines, oxidant-free radicals, inhaled particles, ultraviolet irradiation, and bacterial or viral products. Inappropriate activation of NF-*k*B has been linked to inflammatory events associated with autoimmune arthritis, asthma, septic shock, lung fibrosis, glomerulonephritis, atherosclerosis, and AIDS. In contrast, complete and persistent inhibition of NF- *k*B has been linked directly to apoptosis, inappropriate immune cell development, and delayed cell growth.

NF-*k*B is essential for promoting inflammation-associated cancer and is therefore a potential target for cancer prevention in chronic inflammatory diseases Activation of NF- *k*B, which is frequently detected in tumors, may constitute a missing link between inflammation and cancer. Pikarsky et al. reported that activation of the NF- kB pathway by the TNF receptor EDAR and its downstream adaptor EDARADD is essential for the development of hair follicles, teeth, exocrine glands, and other ectodermal derivatives (71).

In addition, Reuter et al. reported that NF- *k*B1 plays a major role in the expression of pro-inflammatory proteins that lead to increased blood vessel permeability, tissue edema, and pain sensitization that underlie the pathogenesis of migraine and that blockade of NF- *k*B 1 could be a transcriptional target of antimigraine drugs (72).

CREB binding protein (CBP)

The CBP gene is ubiquitously expressed and is involved in the transcriptional co activation of many different transcription factors. The protein encoded by this gene has intrinsic histone acetyltransferase activity and also acts as a scaffold to stabilize additional protein interactions with the transcription complex. This protein acetylates both histone and non-histone proteins.

Mutations in this gene cause Rubinstein-Taybi syndrome (RTS), a multiple congenital anomaly syndrome, characterized by mental retardation. Affected individuals also have an increased risk of tumor formation (73).

Cyclin-dependent kinase inhibitor 1A (p21, Cip1)

This gene encodes a potent cyclin-dependent kinase inhibitor. The expression of this gene is tightly controlled by the tumor suppressor protein p53, through which this protein mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli.

This protein plays a regulatory role in S phase DNA replication and DNA damage repair. It plays a critical role in the cellular response to DNA damage, and it's over expression results in cell cycle arrest. Up regulation of CDKN1A mRNA and protein following ionizing radiation is dependent on p53 and CDKN1A mediates cell cycle arrest in response to the p53 checkpoint pathway (74). Telomere shortening limits the proliferative life span of human cells by activation of DNA damage pathways, including up regulation of the cell cycle inhibitor p21, encoded by the CDKN1A gene. Telomere shortening is associated with impaired organ maintenance and shortened life span in humans (75).

Nucleolar protein 3 (NOL3)

NOL3 (apoptosis repressor with CARD domain) encodes an anti-apoptotic protein that has been shown to down-regulate the enzyme activities of Caspase 2, Caspase 8 and tumor protein p53. Multiple transcript variants encoding different isoforms have been found for this gene (76).

TNF receptor-associated factor 6 (TRAF6)

The protein encoded by this gene is a member of the TNF receptor associated factor (TRAF) protein family. TRAF proteins are associated with, and mediate signal transduction from members of the TNF receptor super family. TRAF6 is a signal transducer in the NF-kappa-B pathway that activates I-kappa-B kinase in response to proinflammatory cytokines.

Interaction with TRAF6 leads to ECSIT ubiquitination and enrichment at the mitochondrial periphery, resulting in increased mitochondrial and cellular ROS generation. ECSIT- and TRAF6-depleted macrophages have decreased levels of TLR-induced ROS and are significantly impaired in their ability to kill intracellular bacteria (77). This is a novel pathway linking innate immune signaling to mitochondria and implicates mROS as an important component of antibacterial responses, and further establishes mitochondria as hubs for innate immune signaling.

Apoptosis-related cysteine peptidase (Caspase 3)

This gene encodes a protein which is a member of the cysteine-aspartic acid protease (Caspase) family. Sequential activation of Caspases plays a central role in the execution-phase of cell apoptosis. It is the predominant Caspase involved in the cleavage of amyloid-

beta 4A precursor protein, which is associated with neuronal death in Alzheimer's disease. Caspases thus appear to play a dual role in proteolytic processing of APP and the resulting propensity for amyloid-beta peptide formation, as well as in the ultimate apoptotic death of neurons in Alzheimer disease. Fernando reported that skeletal muscle differentiation depends on the activity of the key apoptotic protease Caspase-3.

Calmodulin (CALM1)

Calmodulin is the archetype of the family of calcium-modulated proteins. They are identified by their occurrence in the cytosol or on membranes facing the cytosol and by a high affinity for calcium. Calmodulin mediates the control of a large number of enzymes, ion channels and other proteins by Ca (2+). Among the enzymes to be stimulated by the calmodulin-Ca (2+) complex are a number of protein kinases and phosphatases. Together with CEP110 and centrin, is involved in a genetic pathway that regulates the centrosome cycle and progression through cytokinesis. Mototani et al. suggested that the transcriptional level of CALM1 may be associated with susceptibility for hip OA through modulation of chondrogenic activity (78).

Fas (TNFRSF6)-associated via death domain (FADD)

FADD is a universal adaptor protein in apoptosis that mediates signaling of all known death domain-containing members of the TNF receptor super family (79).

The protein encoded by this gene is an adaptor molecule that interacts with various cell surface receptors and mediates cell apoptotic signals. Through its C-terminal death domain, this protein can be recruited by TNFRSF6/Fas-receptor, tumor necrosis factor receptor, TNFRSF25, and TNFSF10/TRAIL-receptor, and thus it participates in the death signaling initiated by these receptors (88). Involved in interferon-mediated antiviral immune response, plays a role in the positive regulation of interferon signaling.

TNF receptor super family, member 6 (FAS R)

The protein encoded by this gene is a member of the TNF-receptor super family. This receptor contains a death domain. It has been shown to play a central role in the physiological regulation of programmed cell death, and has been implicated in the pathogenesis of various malignancies and diseases of the immune system. The interaction of this receptor with its ligand allows the formation of a death-inducing signaling complex that includes Fas-associated death domain protein (FADD), Caspase 8, and Caspase 10. The autoproteolytic processing of the Caspases in the complex triggers a downstream Caspase cascade, and leads to apoptosis. This receptor has been also shown to activate NF-*k*B, MAPK3/ERK1, and MAPK8/JNK. FAS- mediated apoptosis may have a role in the induction of peripheral tolerance, in the antigen-stimulated suicide of mature T-cells, or both. Landau et al. reported that reduced FAS expression increases susceptibility to neuro-degeneration and that FAS has a role in neuro-protection (80).

Phospholipase C, gamma 1 (PLC gamma)

The protein encoded by this gene catalyzes the formation of inositol 1, 4, 5-trisphosphate and diacylglycerol from phosphatidylinositol 4, 5-bisphosphate. This reaction uses calcium as a cofactor and plays an important role in the intracellular transduction of receptor-mediated tyrosine kinase activators. Also, this protein has been shown to be a major substrate for heparin-binding growth factor 1 (acidic fibroblast growth factor)-activated tyrosine kinase. PLC Plays an important role in the regulation of intracellular signaling cascades. It becomes activated in response to ligand- mediated activation of receptor-type tyrosine kinases, such as PDGFRA, PDGFRB, and FGFR1-4. It also plays a role in actin reorganization and cell migration. Chuang et al. has reported that activation of PLC signaling systems regulates other members of the TRP channel family (81).

II. GSTT1*0 Deletions

Gene expression changes following the 4-week blueberry intervention in lymphocytes of subjects bearing GSTT1*0 deletion, mostly involved G-Protein signaling pathways. Biological functions of these pathways are diverse and among the highest ranked by GeneGo Metacore™ (Table 6).
The G-Protein Signaling pathways that were significantly modulated include:

a. Rap2A regulation
b. Rap2B regulation
c. RhoA regulation
d. G-Protein alpha-12 signaling

In the G-protein signaling pathways and cellular processes listed, several genes were modulated which will be summarized below. In addition, gene networks for the immune response biological processes were generated in MetaCore™ in order to visualize the complex set of connections between the genes and their interactions (Figures 5 and 6).

G protein signaling is comprised of G protein coupled receptors (GPCRs) that detect ligands or sense cations, heterotrimeric G proteins, and downstream effectors and regulators. G protein signaling plays important roles in bone development, remodeling, and disease. In human cases, mutations of certain GPCRs and G proteins impair bone development and metabolism, resulting in bone diseases (42).

The metabotropic glutamate receptors (mGluR) consist of a family of eight G-protein-coupled receptors that differ in their function, distribution and physiological roles within the central nervous system. In recent years substantial efforts have been made towards developing selective agonists and antagonists which have proven useful for elucidating their potential as novel targets for the treatment of psychiatric and neurological diseases (43).
In this section, we will review the G-protein Signaling pathways involved and describe the details of modulated genes and their related networks.

a. **G-Protein signaling : Rap2A regulation pathway**

In this study, members of Rap2A regulation pathway were modulated. Rap2A belong to a family of the small GTP-binding proteins (G-proteins) - monomeric G proteins. The Rap subfamily consists of four members: Rap1A, Rap1B, and Rap2A, Rap2B proteins.
Rap2A is localized at the cytoplasmic surface of the plasma membrane. Like other G-proteins, Rap2A has two interconvertible forms: GDP-bound inactive and GTP-bound active forms (24).
Conversion from GDP-bound form to GTP-bound catalyzed by the guanine nucleotide exchange factor (GEF), of which the activity is often regulated by an upstream signal. GEF first interacts with the GDP-bound form and releases bound GDP to form a binary complex of a small G protein and GEP. Then, GEF in this complex is replaced by GTP to form the GTP-bound form.
Proteins named "GTPase activated proteins" (GAP) stimulate that reaction of conversion hydrolysis. Among Rap2A effectors RPIP8 is a specific effector of the Rap2 protein in cells exhibiting neuronal properties. The second messenger cAMP-pathway modulates G-protein activity via Rap2A and PKA (33).

b. G-Protein signaling: Rap2B regulation pathway

In addition to Rap2A regulation, members of Rap2B regulation pathways were also modulated in this study. RAP2B member of RAS oncogene family (Rap-2B) is a member of the Ras-like small GTPases family expressed in platelets. The protein is known to influence platelet activation (34, 35).
Activity of Rap-2B is mainly regulated by guanine nucleotide exchange factors (GEFs) that promote GTP-bound (active) state of Rap-2B. Main GEFs for Rap-2B are Rap guanine nucleotide exchange factors 2 and 5 (PDZ-GEF1 and MR-GEF) (36). Activated Rap-2B can stimulate enzymatic activity of Phospholipase C epsilon 1 (PLC-epsilon) (37).

c. G-Protein signaling: RhoA regulation pathway

In addition to Rap2A and Rap2B regulation pathways, members of RhoA regulation pathway were also modulated in this study. Ras homolog gene family, member A (RhoA) is a member of a family of small GTPases. Rho GTPases control multiple cellular processes, including actin and microtubule dynamics, gene expression, the cell cycle, cell polarity and membrane transport, through their ability to bind to numerous downstream effectors, which lead to diverse parallel downstream signaling pathways (38).
RhoA pathway can be activated by different signaling events that lead to various Rho GEFs activation. Insulin-like growth factor 1 (IGF-1) signaling promotes activation of Insulin-like growth factor 1 receptor (IGF-1 receptor) that forms a complex with Rho guanine nucleotide exchange factor (GEF) 12 (LARG). G-proteins alpha-q/11 and G-protein alpha-12 family can also associate with LARG thus promoting RhoA activation (39). Binding of RhoA to key effectors leads to actin polymerization and cytoskeleton rearrangements.

d. G-Protein signaling : G-Protein alpha-12 signaling pathway

In addition to Rap2A and Rap2B regulation pathways and RhoA regulation pathway, we also noticed that members of alpha 12 signaling pathway were also modulated in this study

.Activation by ligands of G-protein coupled receptors that interact with the trimeric G-protein alpha-12/beta/gamma causes the exchange of GDP for GTP bound to G protein alpha subunits followed by dissociation of the beta/gamma heterodimers. Free alpha and beta/gamma subunits are active and transmit signals into the cells.
G-protein alpha-12 family subunits directly bind to and stimulate Ras GTPase-activating protein (RASA2). RASA2 hydrolyzes the bound GTP of the small GTP-bound GTPase to produce the GDP-bound form of the protein. This conversion inhibits small GTPases M-Ras, R-Ras and TC21 (32).R-Ras is implicated in regulation of various cell functions, such as gene expression and cell proliferation via the activation of the c-Raf-1/ Mitogen-activated protein kinase kinase 1 and 2 (MEK1 and MEK2)/ Mitogen-activated protein kinases 1 and 3 (ERK1/2) signaling pathway (33).

In the G-protein signaling cellular processes impacted by the 4-week blueberry dietary intervention, several genes were modulated (Table 7) for which we include a brief summary of each gene below. Overall, an up regulation of Phospholipase D1, phosphatidylcholine-specific(PLD1) and Insulin-like growth factor 1 receptor (IGF-1 receptor) have been observed, while a down regulation of Mitogen-activated protein kinase 1 MEKK1(MAP3 K1), PTK2 protein tyrosine kinase 2 (FAK1), Solute carrier family 8 (sodium/calcium exchanger), member 1(NCX1), Myocyte enhancer factor 2A (MEF2A), Protein kinase C, beta 1 (PKC-beta1), Muscle RAS oncogene homolog (M-Ras), Rho guanine nucleotide exchange factor (GEF) 12 (LARG) and RAS p21 protein activator 2 (RASA2) were observed in this study.

Furthermore, in order to visualize the complex set of connections between the genes and their interactions two gene networks for the G-protein Signaling pathways were generated in MetaCore^{TM} as identified by GeneGo analysis using shortest paths networks. The network of genes including MEKK1 (MAP3K1), FAK1, NCX1, MEF2A and PKC-beta are involved in intracellular signal transduction, wound healing, platelet activation, regulation of body fluid levels and response to external stimulus. The network of genes including M-Ras, PLD1, LARG, IGF-1 receptor and RASA2 are involved in response to peptide hormone and endogenous stimulus, in response to organic substance and in nerve growth factor receptor signaling pathway (Figures 5 and 6).

Mitogen-activated protein kinase 1 MEKK1 (MAP3 K1)

The protein encoded by this gene is a serine/threonine kinase and is part of some signal transduction cascades, including the ERK and JNK kinase pathways as well as the NF-kappa-B pathway. The encoded protein is activated by autophosphorylation and requires magnesium as a cofactor in phosphorylating other proteins. MAP3K1, or MEKK1, is a mitogen-activated protein kinase (MAPK) kinase kinase that regulates the ERK and JNK in MAPK pathways, as well as the transcription factor NF-kappa-B and the transcriptional coactivator p300. MAP3K1 generates antiapoptotic signaling as a full-length protein, but it induces apoptosis following cleavage by Caspases (89).

PTK2 protein tyrosine kinase 2 (FAK1)

This gene encodes a cytoplasmic protein tyrosine kinase which is found concentrated in the focal adhesions that form between cells growing in the presence of extracellular matrix constituents. The encoded protein is a member of the FAK subfamily of protein tyrosine kinases but lacks significant sequence similarity to kinases from other subfamilies. Activation of this gene may be an important early step in cell growth and intracellular signal transduction pathways triggered in response to certain neural peptides or to cell interactions with the extracellular matrix. Non-receptor protein-tyrosine kinase implicated in signaling pathways involved in cell motility, proliferation and apoptosis. Activated by tyrosine-phosphorylation in response to either integrin clustering induced by cell adhesion or antibody cross-linking, or via G-protein coupled receptor (GPCR) occupancy by ligands such as bombesin or lysophosphatidic acid, or via LDL receptor occupancy. It plays a potential role in oncogenic transformations resulting in increased kinase activity. Wong et al. has showed that Fak was required for scar formation after wounding (90).

Solute carrier family 8 (sodium/calcium exchanger), member 1(NCX1)

In cardiac myocytes, Ca (2+) concentrations alternate between high levels during contraction and low levels during relaxation. The increase in Ca (2+) concentration during contraction is primarily due to release of Ca (2+) from intracellular stores. However, some Ca (2+) also enters the cell through the sarcolemma (plasma membrane). During relaxation, Ca (2+) is sequestered within the intracellular stores. To prevent overloading of intracellular stores, the Ca (2+) that entered across the sarcolemma must be extruded from the cell. The Na (+)-Ca (2+) exchanger is the primary mechanism by which the Ca (2+) is extruded from the cell during relaxation. In the heart, the exchanger may play a key role in digitalis action. The exchanger is the dominant mechanism in returning the cardiac myocyte to its resting state following excitation (91).

Myocyte enhancer factor 2A (MEF2A)

The protein encoded by this gene is a DNA-binding transcription factor that activates many muscle-specific, growth factor-induced, and stress-induced genes. The encoded protein can act as a homodimer or as a heterodimer and is involved in several cellular processes, including muscle development, neuronal differentiation, cell growth control, and apoptosis. It is also involved in the activation of numerous growth factor- and stress-induced genes. It mediates cellular functions not only in skeletal and cardiac muscle development, but also in neuronal differentiation and survival. It plays diverse roles in the control of cell growth, survival and apoptosis via p38 MAPK signaling in muscle-specific and/or growth factor-related transcription. Wang et al. detected expression of MEF2A protein in nuclei of human proliferating smooth muscle cells. An increased smooth muscle cell proliferation is associated with accelerated atherosclerosis (92).

Protein kinase C, beta 1 (PKC-beta1)

PRKCB1 is a member of the protein kinase C (PKC) gene family. The 66-kD isoform of the growth factor adaptor SHC, p66 (SHC), translates oxidative damage into cell death by acting as a reactive oxygen species producer within mitochondria.

Pinton et al. demonstrated that protein kinase C-beta, activated by oxidative conditions in the cell, triggers mitochondrial accumulation of the protein after it is recognized by the prolyl isomerase PIN1 (93). Once imported, p66 (SHC) causes alterations of mitochondrial calcium ion responses and 3-dimensional structure, thus causing apoptosis. Pinton identified a signaling route that activates an apoptotic inducer shortening the life span.

Muscle RAS oncogene homolog (M-Ras)

M-Ras gene encodes a member of the Ras family of small GTPases. The membrane-associated proteins function as signal transducers in multiple processes including cell growth and differentiation, and deregulation of Ras signaling has been associated with many types of cancer. The encoded protein may play a role in the tumor necrosis factor-alpha and MAP kinase signaling pathways.
M-Ras may serve as an important signal transducer for a novel upstream stimuli in controlling cell proliferation. Weakly activates the MAP kinase pathway.

Phospholipase D1, phosphatidylcholine-specific (PLD1)

This gene encodes a phosphatidylcholine-specific phospholipase which catalyzes the hydrolysis of phosphatidylcholine in order to yield phosphatidic acid and choline. The enzyme may play a role in signal transduction and sub cellular trafficking.
PLDs catalyze the hydrolysis of PC to produce phosphatidic acid and choline. A range of agonists acting through G protein-coupled receptors and receptor tyrosine kinases stimulate this hydrolysis. PC-specific PLD activity has been implicated in numerous cellular pathways, including signal transduction, membrane trafficking, and the regulation of mitosis (94).
Cai et al. found that PLD1 enzymatic activity was decreased in N2a cells with familial Alzheimer disease-3 (AD3). His findings showed that PLD1 regulates intracellular trafficking of beta-amyloid, distinct from its effect on gamma-secretase activity (95).

Rho guanine nucleotide exchange factor (GEF) 12 (LARG)

Rho GTPases play a fundamental role in numerous cellular processes that are initiated by extracellular stimuli working through G protein-coupled receptors. The encoded protein may form a complex with G proteins and stimulate Rho-dependent signals. This protein has been observed to form a myeloid/lymphoid fusion partner in acute myeloid leukemia.

Insulin-like growth factor 1 receptor (IGF-1 receptor)

This receptor binds insulin-like growth factor with a high affinity. It has tyrosine kinase activity. The insulin-like growth factor I receptor plays a critical role in transformation events. Cleavage of the precursor generates alpha and beta subunits. It has a tyrosine-protein kinase activity, which is necessary for the activation of the IGF1-stimulated downstream signaling cascade. It is highly over expressed in most malignant tissues where it functions as an anti-apoptotic agent by enhancing cell survival. All-Ericsson et al. focusing on its role in cell growth in uveal melanoma suggested a significant association between high IGF1R expression and death due to metastatic disease. Because IGF1R is produced mainly in the

liver, the preferential site for uveal melanoma metastases, these results pointed to the possibility of interfering therapeutically with IGF1R in uveal melanoma (102).

RAS p21 protein activator 2 (RASA2)

The protein encoded by this gene is member of the GAP1 family of GTPase-activating proteins. The gene product stimulates the GTPase activity of normal RAS p21 but not its oncogenic counterpart acting as a suppressor of RAS function, the protein enhances the weak intrinsic GTPase activity of RAS proteins resulting in the inactive GDP-bound form of RAS, thereby allowing control of cellular proliferation and differentiation.
In addition, gene networks for the G-protein Signaling pathways, identified by MetacoreTM GeneGo analysis using shortest paths networks, are generated in MetaCore^{TM} in order to visualize the complex set of connections between the genes and their interactions (Figures 5, 6).

XRCC1*4 Variants:

In contrast to GSTT1 variants, fewer biological pathways were found to be significantly ($p < 0.05$) affected in the XRCC1 variants. Furthermore, the numbers of significantly modulated genes in the pathways were low.

In the XRCC1*4 wildtype variants, the most significant pathways found for the correlating genes are related to the cellular processes immune response whereas in the XRCC1*4 heterozygous and homozygous variants, pathways modulations related to glutathione metabolism cellular processes are mainly observed (Table 7).

Network of modulated genes was identified for XRCC1*4 variant using GeneGo shortest path networks. The significant networks of modulated genes consisted of regulation of metabolic and biological process, innate immune response, response to stress and wounding, signal transduction and regulation of protein metabolic processes. Whereas in XRCC1*4 heterozygous and homozygous variants, pathways modulations mainly related to glutathione metabolism cellular processes are observed (Table 8).

The results at the level of pathways were visualized using Venn diagram of GeneGo biological pathways. Overall comparison of the results between the paired t-test and correlation analyses of significantly modulated pathways for XRCC1*4 wild type and XRCC1*4 Homozygous and Heterozygous variants for the top twenty significant pathways as ranked by GeneGo Metacore^{TM}, showed no similarity at the level specific biological pathways (Figure 2).

III. XRCC1*4 Wildtypes

For the XRCC1*4 wild types variants, the most significant pathways for the correlating genes were among the immune response cellular processes.

Gene expression changes following the 4-week blueberry intervention in lymphocytes of subjects bearing XRCC1*4 wt, involved a few immune response signaling pathways. In addition, we observed some modulation of pathways related to Glutathione metabolism. The glutathione metabolism pathway modulation was also found among the significant pathways in the XRCC1*4 heterozygote and homozygote variant subgroup hence we will only review this pathway when we discuss the blueberry intervention impact on heterozygote and homozygote variants. Biological functions of selected pathways are diverse and among the highest ranking by GeneGo MetacoreTM (Table 7). Significant Immune Response Signaling pathways that were modulated include:

a) TLR signaling pathways
b) CD137 signaling in immune cell
c) IL7 signaling in T lymphocytes

In this section, we will review the Immune response signaling pathways involved and describe the details of modulated genes and their related networks.

a. Immune response-TLR signaling pathways

In the present study, members of TLR signaling pathways were modulated significantly.
In the TLR signaling pathway, both gram-positive and gram-negative bacteria and their cell wall components activate innate immune system of the host and induce secretion of proinflammatory molecules, mainly chemokines and cytokines (44). Toll-like receptors (TLRs) initiate signaling cascades through recognition of a variety of microbial components, thus serving as an important link between innate and adaptive immune responses.
The main TLR-mediated immune response pathway, which is common for all TLRs, is Myeloid differentiation primary response gene 88 (MyD88)-dependent activation of Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor (NF-*k*B) and c-Jun, transcription regulators of number of chemokines and cytokines responsible for cellular immune response. TLR family members can induce autophagy through direct activation of Beclin 1 by MyD88 (45).

b. **Immune response-CD137 signaling in immune cell**

Besides the TLR-signaling pathway, we have seen members of CD137 signaling pathway to be modulated in this study. CD137 (TNFRSF9) is an inducible T cell surface receptor belonging to the tumor necrosis factor receptor super family. It presents on the surfaces of activated CD4+ and CD8+ T cells, monocytes and B lymphocytes. CD137 (TNFRSF9) signaling is activated by binding to its high-affinity ligand CD137 ligand (TNFSF9) expressed on the antigen-presenting cell surface. CD137 (TNFRSF9) signaling promotes cell growth; T cell differentiation and survival (see anti-apoptosis of immune cells (46).
CD137 (TNFRSF9) mediates its action via association with TRAF1 and TRAF2. CD137 (TNFRSF9) binding to TRAF2 leads to activation of the NIK (MAP3K14). NIK (MAP3K14)

activates NF-*k*B via the IKK-alpha/ NF- *k*B IA pathway. NF- *k*B induces transcription of Bcl-XL, Bcl-2 and BFL1, and thus promotes survival (see anti-apoptosis of immune cells (47). CD137(TNFRSF9)-induced ERK1/2 activation also leads to Bim phosphorylation by ERK1/2. It promotes proteosomal degradation of Bim and leads to immune cell survival (48).

c. **Immune response-IL7 signaling in T lymphocytes**

Besides the TLR and CD137 signaling immune response pathways, we have noticed that members of IL7 signaling in lymphocytes were also modulated in this study. IL-7 is an essential cytokine for proliferation, maintenance and survival of T-lymphocytes. IL-7 receptor activates pathways that regulate lymphocyte survival, glucose uptake, proliferation and differentiation (41). Activated IL-7 receptor stimulates Phosphatidylinositol 3-kinase (PI3K)/ V-akt murine thymoma viral oncogene (AKT/PKB) cascade via JAKs/ Insulin receptor substrates 1 and 2 (IRS-1, IRS-2) .AKT/PKB complex induces Solute carrier family 2 member 1 (GLUT1) activation inhibition of transcriptional factor Forkhead box O3 (FOXO3A). GLUT1 stimulates Glucose uptake thus promoting cell survival (50).

IL-7 stimulates V(D)J recombination of T-cell receptor gamma chain (TCR gamma locus) in T cells. STAT5 plays a key role in synthesis and V(D)J recombination of TCR gamma locus with participation of histone acetylases p300 and CBP . It is possibly, that AKT/PKB-dependent inhibition Forkhead box O1 (FKHR) stimulates Recombination activating gene 1 and 2 (RAG1 and RAG2) transcription, thus inducing V(D)J recombination of TCR gamma locus during lymphocyte development (51).

In the immune response signaling pathways , impacted by the 4-week blueberry dietary intervention , listed above for XRCC1*4 wt , several genes were modulated which will be summarized below. (Table 7)

Overall, an up regulation of Toll-like receptor 4(TLR4), BCL2-like 11 (apoptosis activator)(Bim), V-rel reticuloendotheliosis viral oncogene homolog B RelB (NF-*k*B subunit), CREB binding protein (CBP), Myeloid differentiation primary response gene (88) (MyD88), and Mitogen-activated protein kinase kinase 6-MEK6(MAP2K6) have been observed. While a down regulation of BCL2-like 11 (apoptosis facilitator) (Bcl-2), Activating transcription factor 2 (ATF-2), Lymphocyte-specific protein tyrosine kinase (LCK), Phospholipase D1, and phosphatidylcholine-specific (PLD1) were observed in this study.

Furthermore, in order to visualize the complex set of connections between the genes and their interactions two gene networks for the immune response pathways were generated in MetaCore^{TM} and identified by GeneGo analysis using shortest paths networks. The network of genes including Bcl-2, TLR4, CBP, RelB (NF-*k*B subunit) and Bim, involved in positive regulation of metabolic process, positive regulation of biological process and innate immune response. The network of genes including ATF-2, MyD88, Lck, MEK6 (MAP2K6) and PLD1 is involved in response to stress, response to wounding, immune response-activating signal transduction, immune response-regulating signaling pathway and in regulation of protein metabolic process (Figures 7 and 8).

BCL2-like 11 (apoptosis facilitator) (Bcl-2)

BCL-2 family members form hetero- or homodimers and act as anti- or pro-apoptotic regulators that are involved in a wide variety of cellular activities. The protein encoded by this gene contains a Bcl-2 homology domain 3 (BH3). It has been shown to interact with other members of the BCL-2 protein family, including BCL2, BCL2L1/BCL-X(L), and MCL1, and to act as an apoptotic activator.
The expression of this gene can be induced by nerve growth factor (NGF), as well as by the forkhead transcription factor FKHR-L1, which suggests a role of this gene in neuronal and lymphocyte apoptosis (52).

Toll-like receptor 4(TLR4)

The protein encoded by this gene is a member of the Toll-like receptor (TLR) family which plays a fundamental role in pathogen recognition and activation of innate immunity. TLRs recognize pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity.
This receptor is most abundantly expressed in placenta, and in myelomonocytic subpopulation of the leukocytes. It cooperates with LY96 and CD14 to mediate the innate immune response to bacterial lipopolysaccharide (LPS). Acts via MYD88, TIRAP and TRAF6, leading to NF-kappa-B activation, cytokine secretion and the inflammatory response. Age-related macular degeneration 10 (ARMD10), a variant in the TLR4 gene is associated with susceptibility to age-related macular degeneration (53).

CREB binding protein (CBP)

CREB-binding protein, also known as CREBBP or CBP, is a protein that in humans is encoded by the CREBBP gene. The CREB protein carries out its function by activating transcription, where interaction with transcription factors is managed by one or more CREB domains. This gene is involved in the transcriptional co activation of many different transcription factors.
This gene is known to play critical roles in embryonic development, growth control, and homeostasis by coupling chromatin remodeling to transcription factor recognition. The protein encoded by this gene has intrinsic histone acetyltransferase activity and also acts as a scaffold to stabilize additional protein interactions with the transcription complex. Pasqualucci et al. reported that the 2 most common types of B cell non-Hodgkin lymphoma , follicular lymphoma and diffuse large B-cell lymphoma, harbor frequent structural alterations inactivating CREBBP (96). In addition, Mullighan et al. found that 18.3% of relapse cases of acute lymphoblastic leukemia had sequence or deletion mutations in CREBBP and that these frequently occurred in the histone acetyltransferase domain(97).

V-rel reticuloendotheliosis viral oncogene homolog B RelB (NF-*k*B subunit)

Nuclear factor of kappa light polypeptide gene enhancer in B-cells 3 (NF-kappa-B) is a pleiotropic transcription factor which is present in almost all cell types and is involved in many biological processed such as inflammation, immunity, differentiation, cell growth, tumor genesis and apoptosis.

NF-kappa-B is controlled by various mechanisms of post-translational modification and sub cellular compartmentalization as well as by interactions with other cofactors or corepressors. NF-kappa-B complexes are held in the cytoplasm in an inactive state complexed with members of the NF- kappa-B inhibitor (I-kappa-B) family. Vaira et al. found RELB was required for Rankl-induced osteoclastogenesis in vitro and for TNF -induced bone resorption in vivo. He reported that the alternative NF-*k*B pathway, via RELB, plays an essential and unique role in RANKL signaling toward osteoclast development (98).

BCL2-like 11 (apoptosis facilitator)(Bim)

BIM Induces apoptosis. It has been shown to interact with other members of the BCL-2 protein family, including BCL2, BCL2L1/BCL-X(L), and MCL1, and to act as an apoptotic activator. The expression of this gene can be induced by nerve growth factor (NGF), as well as by the forkhead transcription factor FKHR-L1, which suggests a role of this gene in neuronal and lymphocyte apoptosis.

Activating transcription factor 2 (ATF-2)

ATF2 gene encodes a transcription factor that is a member of the leucine zipper family of DNA binding proteins. This protein binds to the cAMP-responsive element (CRE), an octameric palindrome. It forms a homodimer or a heterodimer with c-Jun and stimulates CRE-dependent transcription. This protein is also a histone acetyltransferase (HAT) that specifically acetylates histones H2B and H4 in vitro; thus it may represent a class of sequence-specific factors that activate transcription by direct effects on chromatin components.

Myeloid differentiation primary response gene (88) (MyD88)

This gene encodes a cytosolic adapter protein that plays a central role in the innate and adaptive immune response. This protein functions as an essential signal transducer in the interleukin-1 and Toll-like receptor signaling pathways. T Acts via IRAK1, IRAK2, IRF7 and TRAF6, leading to NF-kappa-B activation, cytokine secretion and the inflammatory response.

These pathways regulate activation of numerous proinflammatory genes. The encoded protein consists of an N-terminal death domain and a C-terminal Toll-interleukin1 receptor domain. Patients with defects in this gene have an increased susceptibility to pyogenic bacterial infections.

Lymphocyte-specific protein tyrosine kinase (LCK)

This gene is a member of the Src family of protein tyrosine kinases (PTKs). The encoded protein is a key signaling molecule in the selection and maturation of developing T-cells. The protein localizes to the plasma membrane and pericentrosomal vesicles, and binds to cell surface receptors, including CD4 and CD8, and other signaling molecules.

It plays a key role in T-cell antigen receptor (TCR)-linked signal transduction pathways. Associated with the cytoplasmic portions of the CD4 and CD8 surface receptors, once stimulated, the TCR recruits the tyrosine kinase ZAP70, which becomes phosphorylated and activated by LCK. Following this, a large number of signaling molecules are recruited, ultimately leading to lymphokine production. LCK also contributes to signaling by other receptor molecules. It also plays a role in the IL2 receptor-linked signaling pathway that controls the T-cell proliferative response (99).

Mitogen-activated protein kinase kinase 6-MEK6(MAP2K6)

Mitogen-activated protein kinases (MAPKs) are key components in various cellular signal transduction pathways that affect growth factor-induced proliferation, gene expression, and compensation for environmental changes.
This gene encodes a member of the dual specificity protein kinase family, which functions as a mitogen-activated protein (MAP) kinase kinase. MAP kinases, also known as extracellular signal-regulated kinases (ERKs), act as an integration point for multiple biochemical signals. This protein phosphorylates and activates p38 MAP kinase in response to inflammatory cytokines or environmental stress. As an essential component of p38 MAP kinase mediated signal transduction pathway, this gene is involved in many cellular processes such as stress induced cell cycle arrest, transcription activation and apoptosis.
MAP2K6/MKK6 is the major MAPK11 activator in response to TNF. MAP2K6/MKK6 mediates apoptotic cell death in thymocytes. It acts also as a regulator for melanocytes dendricity, through the modulation of Rho family GTPases.

Phospholipase D1, phosphatidylcholine-specific (PLD1)

This gene encodes a phosphatidylcholine-specific phospholipase which catalyzes the hydrolysis of phosphatidylcholine in order to yield phosphatidic acid and choline. The enzyme may play a role in signal transduction and sub cellular trafficking. Implicated as a critical step in numerous cellular pathways, including signal transduction, membrane trafficking, and the regulation of mitosis (100).

IV. XRCC1*4 Heterozygotes and Homozygotes

MetaCore GeneGo analysis of pathways and cellular processes for Wilms study subjects following the 4-week blueberry intervention, showed that few pathways were significantly ($p < 0.05$) modulated in XRCC1*4 heterozygote and homozygote variants (Table 6). The modulated pathways were found to be part of the Glutathione Metabolism cellular processes (Figures 9).

Glutathione Metabolism

In both the XRCC1*4 wt and heterozygote and homozygote variants in this study, members of Glutathione Metabolism pathways were shown to be modulated. In this section, we will briefly discuss this pathway.

Human glutathione S-transferases (GST), mainly known as phase II enzymes, are involved in the detoxification of endogenous and exogenous electrophilic compounds and play an important role in protection against oxidative stress.

Glutathione disulfide can be formed directly from Glutathione in the reaction catalyzed by Glutathione peroxidase 4 (GPX4 (PHGPx), Glutathione peroxidase 1 (GPX1), Glutathione peroxidase 2 (GPX2) (19), Glutathione peroxidase 3 (plasma) (GPX3), Glutathione peroxidase 6 (GPX6), Glutathione peroxidase 7 (GPX7), Glutathione peroxidase 5 (GPX5) and GSHR. (20).These reactions produce R-S-Glutathione (glutathione conjugated to a moiety) products.

Polymorphisms in the GSTT gene families which are involved in detoxification and antioxidative mechanisms have been associated with alterations in cancer risk for several different cancers (23).

As for the XRCC1*4 wt heterozygote and homozygote variants, we report that a few genes, involved in glutathione metabolism, were significantly modulated. Study results show that GGT1, GSTM2 homodimer, GSTM3, GSTA4, GPX7 and GSTA2 homodimer, were all up - regulated while GCL cat, was down-regulated (Table 7).

Discussion

To our knowledge, this is the first research of whole genome gene expression analysis together with the impact of genetic polymorphism following a dietary intervention in humans. Additionally, this is a unique unparalleled study by which a comparison is made between the biological pathways affected by a 4-week blueberry dietary intake on different polymorphisms of GSTT1 and XRCC1. Moreover, the effects of on immune response cellular processes, G-protein signaling and glutathione Metabolism, which may be contributing to health benefits of blueberries in humans, are discussed here for the first time.

Our data suggests that dietary supplementation with flavonoid-rich foods, such as blueberry has an impact on biological pathways and cellular processes in which oxidative cell damage plays a role, such as neuro degenerative disease, cancer or cardiovascular disease. Furthermore, mechanisms underpinning berry's ability to induce improvements in health are linked to the potential of absorbed flavonoids and their metabolites to interact with and modulate critical signaling pathways, transcription factors and gene and/or protein expressions. We will compare and discuss each subgroup separately in the following section.

GSTT1* 0 Wildtypes:

Overall in the GSTT1*0 wt variant genotype, we identified 2 important network of genes.

The network of genes including p21, CBP, TRAF6, RelB (NF-*k*B subunit) and NOL3 described in the result section seem to be involved in regulation of cell death and apoptosis. In this network, we noticed that NF-*k*B has been up-regulated. Up regulation of RELB, one of the NF-*k*B members could mean modulation of expression of genes encoding for different IkB proteins. Also CBP and P21 both part of IFN gamma signaling - immune response pathway have been up-regulated. Up regulation of P21 gene could imply a shortening of telomeres and impact on aging process and its related disease. Lastly we have seen a down-regulation of TRAF6 and an an up-regulation of TLR4 in HGMB1/TLR signaling - immune response pathway. This results in increased mitochondrial and cellular ROS generation. Apoptosis proceeded by increased generation of reactive oxygen species, and associated with p21 activation, increased expression of CBP, and an up-regulation of NOL3 in immune response and over expression of NF-*k*B and NOL3, could imply a less protective effect against oxidative damage.

The network of genes including Calmodulin, Caspase-3, FasR(CD95), PLC-gamma 1 and FADD are involved in lipid catabolic process, phospholipid metabolic process, organophosphate metabolic process, positive regulation of molecular function and platelet activation. In this network, our findings suggest an up -regulation of Caspase 3. Activation of CASP3 leads to the ultimate demise of the cell. No genes which play a role in inhibition of caspases were modulated; thereby a caspase cascade ultimately leading to initiation of

apoptosis is implied here. We have also noticed an up-regulation of CALM1 in the immune response- CD16 signaling in NK cell pathway which may be associated with susceptibility for Osteoarthritis. In addition, a down-regulation of PLC gamma which has an important role in the regulation of other members of the TRP channels family. Lastly down-regulation of FADD and FAS R has been reported. Since FAS has a role in neuroprotection (101), a Reduced FAS expression could imply increase in susceptibility to neurodegeneration.

Overall, we illustrate here that the blueberry juice dietary intervention has a complex and strong effect on immune response related pathways in this subgroup. Apoptosis in lymphocytes of subjects carrying the GSTT1*0 wt genotype may describe a possible sequence of molecular events underlying its lethal effect, and suggests that it may prove useful in therapies of cancer and aging related diseases.

GSTT1* 0 Deletions:

Overall in the GSTT1* 0 deletion variant genotype, we identified 2 important G-protein signaling networks of genes.

The network of genes including MEKK1 (MAP3K1), FAK1, NCX1, MEF2A and PKC-beta described above seem to be involved in intracellular signal transduction, wound healing, platelet activation, regulation of body fluid levels and response to external stimulus. In this network, we have reported a down-regulation of MEKK1 in the G-protein alpha 12 signaling pathway. This gene is thought to be associated with breast cancer and this locus interacts multiplicatively on breast cancer risk in BRCA2 mutation carriers (82) hence the down regulation may imply that carriers of this gene may be at a slightly lower risk of breast cancer.

There are certainly many factors contributing to the rise in heart disease, poor diet likely being the most important. Studies have suggested that blueberries could strengthen blood vessels against oxidative stress that may lead to heart disease. In this study we have seen a down-regulation of FAK1, NCX1, PKC beta 1 and MEF 2A. This could imply a cardiovascular benefit for subjects with the GSTT1*0 deletion genotype. In addition the results of recent study published berry to lowering cholesterol and neurodegenerative diseases like Alzheimer (62).

The network of genes including M-Ras, PLD1, LARG, IGF-1 receptor and RASA2 are involved in response to peptide hormone and endogenous stimulus, in response to organic substance and in nerve growth factor receptor signaling pathway. Our findings suggest a down-regulation of M-Ras in the G-protein-RAP2A regulation pathway. Since deregulation of Ras signaling has been associated with many types of cancer (83), this could imply an involvement in control of cancer cell proliferation .

Also a down-regulation of LARG which has a fundamental role in numerous cellular processes that are initiated by extracellular stimuli working through G protein-coupled receptors. In addition a down -regulation of (RASA2) involved in the G-protein-alpha -12

signaling pathway. RASA2 acts as a suppressor of RAS function, the protein enhances the weak intrinsic GTPase activity of RAS proteins thereby its down regulation may cause inhibition of the control of cellular proliferation and differentiation. Recent research shows that the IGF-1 receptor gene is involved in growth and has also shown involvement in longevity (84).

Lastly we have observed an up-regulation of PLD1 in the G-protein-Rho a regulation pathway. This could imply an increased involvement in regulation of intracellular trafficking, signal transduction, membrane trafficking, and the regulation of mitosis.

Overall, the 4-week blueberry -apple juice dietary intervention has shown a complex effect on G-protein signaling related pathways in this subgroup. The control of cell proliferation is of central importance to the proper development of a multi-cellular organism, the homeostatic maintenance of tissues, and the ability of certain cell types to respond appropriately to environmental cues. Disruption of normal cell growth control underlies many pathological conditions, including endothelial proliferative disorders in cardiovascular disease and neurodegenerative disease as well as the development of tumors. Therefore the dietary blueberry intake may prove to be protective for the subjects carrying the GSTT1* deletion variants.

XRCC1*4 Wildtypes, Homozygous and Heterozygous:

With regards to XRCC1*4 wt variant, we identified two immune response networks of genes that were significantly modulated following the 4-weeks of blueberry juice intervention.

The network of genes including Bcl-2, TLR4, CBP, RelB (NF-*k*B subunit) and Bim, involved in positive regulation of metabolic process, positive regulation of biological process and innate immune response.

Our findings suggest that the anti-apoptotic genes BCL2, involved in the CD137 signaling in immune cell pathway were down regulated in the present study, thereby abolishing their inhibiting effects on apoptosis. Also ATF2 which is a transcription factor that binds to the cAMP-responsive element (CRE) was down-regulated. This down regulation may imply a negative control on transcription by direct effects on chromatin components.

BIM, which is also involved in the CD137 signaling pathway in immune cells, was up regulated. BIM is known to induce apoptosis and interact with other members of the BCL-2 protein family as an apoptotic activator. This suggests an activated role in neuronal and lymphocyte apoptosis.
TLR4, involved in the TLR signaling pathway was up-regulated in the present study. Also NF-*k*B which is involved in the pathway was up regulated. Modulation of Nf-κB gene expression or protein levels by blueberries were reported in a few in vitro studies in the past (85).

To our knowledge, this is the first human study in which Nf-κB is reported to be modulated after an intervention with blueberries. The positive correlation found here, can mean a less protective effect against ex vivo induced oxidative DNA damage as it depicts the role of Nf-κB in the response to exposure to reactive oxygen species. Additionally, CREB binding protein (CBP) was up regulated. This over expression may result in an increase of apoptotic activity and cell cycle arrest by stimulation of p53. This is an important gene which is known to have a role in Non-Hodgkin Lymphoma and in Acute Lymphoblastic Leukemia (86).

The network of genes including ATF-2, MyD88, Lck, MEK6 (MAP2K6) and PLD1 is involved in response to stress, response to wounding, immune response-activating signal transduction, immune response-regulating signaling pathway and in regulation of protein metabolic process.

Our findings suggest that PLD1 was down-regulated. The enzyme may play a role in signal transduction and sub cellular trafficking. Its down regulation can be considered a critical step in numerous cellular pathways, including signal transduction, membrane trafficking, and the regulation of mitosis. In addition LCK which is involved in the IL7 signaling pathway in T-lymphocytes was down-regulated. While, MYD88 involved in the TLR signaling pathway in immune response cell was up- regulated. This over expression could lead to NF-*k*B activation. Lastly, our findings suggest that MAP2K6 was up-regulated. This gene is involved in many cellular processes such as stress induced cell cycle arrest, transcription activation and apoptosis hence its up regulation may have an important role in inducing apoptosis (87).

It appears that blueberry intervention has caused a complex effect on immune response. In addition, intervention effects have also been observed on glutathione metabolism pathways in XRCC1*4 wt variants. Further studies to elicit these effects in details are needed.

In the XRCC1*4 heterozygote and homozygote subgroup, the blueberry intervention modulated a few genes involved in glutathione metabolism. Glutathione plays important roles in antioxidant defence, nutrient metabolism, and regulation of cellular events (including gene expression, DNA and protein synthesis, cell proliferation and apoptosis, signal transduction, cytokine production and immune response, and protein glutathionylation) and Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many diseases (such as seizure, Alzheimer's disease, Parkinson's disease, liver disease, cystic fibrosis, sickle cell anaemia, HIV, AIDS, cancer, heart attack, stroke, and diabetes (58).

The correlation of glutathione S-transferase (GST) T1 genetic polymorphisms with oxidative stress-related chronic diseases was proven recently (21, 22). Detoxification of exogenous harmful compounds (such as smoking of cigarettes and consumption of alcohol) often occurs by phase II enzymes such as GSTs. Proper functioning of these enzymes may be deficient due to the presence of particular genetic polymorphisms in these GSTs. Recent studies have reported associations between head and neck squamous cell carcinoma (HNSCC) and allelic variants of glutathione S-transferase T1 (GSTT1) in head and neck cancer patients (23, 55). Moreover, in recent studies the GSTT1 genes were screened for their functional impact on concerned proteins and their plausible roles in breast cancer susceptibility (55) and in lung cancer (56)
DNA damage contributes to the mechanisms of aging and disease. It has broad relevance to human pathobiology with its involvement in cancer, aging and elevated DNA damage is

found in several neurodegenerative disorders such as Alzheimer and Parkinsons (57). Endogenous and environmental agents can cause DNA damage in cells. Dietary mutagenic chemicals, ultraviolet and ionizing radiation, and heavy metals are environmental agents that damage the genome, causing DNA cross-links, adducts, and oxidative cleavage. The x-ray repair cross-complementing protein 1 (XRCC1) protein plays a major role in facilitating the repair of single-strand breaks in human cells, via an ability to interact with multiple enzymatic components of repair reactions (58)
XRCC1 has been reported to be associated with several different diseases including glaucoma which have been shown to be dependent upon the genetics and environmental factors (59). Degenerative ocular diseases are widespread in the population and represent a major cause of reversible and irreversible blindness. Scientific evidences have been accumulating supporting the role of genotoxic damage and gene environment interactions in the pathogenesis of these diseases including glaucoma, age-related macular degeneration (AMD), and cataract in which there seems to be high susceptibility to oxidative stress observed in certain population subgroups (60).

Overall, from the results of biological pathway analysis in this study, we are able to conclude that the group of subjects with XRCC1*4 heterozygote and homozygote genotype may not have the added-value of having a significant modulation in the immune related pathways as it has been seen in the XRCC1*4 wt subgroup. The impact on glutathione metabolism pathway which is observed in heterozygote and homozygote, was also seen in the XRCC1*4 wildtypes hence the wildtype variants are expected to experience a more chemoprotective effect of blueberries if a diet rich in these fruits is consumed.

Investigation of the modifications of gene expression level following the blueberry intervention in this study provides an update on the mechanism of action of blueberry intervention in human subjects. In this study we have investigated markers of oxidative stress linked to gene expression modulation at the whole genome level. Molecular pathways and target genes, associated with GSTT1 and XRCC1 polymorphisms, which contribute to chemopreventive effects of blueberry in human individuals, reported here for the first time, will help us comprehend the pathways associated with changes in phenotypic biomarkers of disease risk.

Studies with fruits and vegetables have reported that null genotypes of GSTT1 result in a complete lack of their respective enzymes and considerable variability in the modifying effect of GST genotypes on cancer risk (66). Biologic response may vary due to genetic and other factors that influence exposure, metabolism and disposition of the fruit, and interaction with target genes (67). Moreover, fruit exposure in-vivo may be influenced by the environment in the digestive tract (e.g., hydrolysis by gut microbiota, microbiota composition, pH, nutrient interactions, etc.), and genetic variation in enzymes involved in metabolism. These factors may translate into Interindividual differences in the protective effects of blueberries (68, 69 and 70).

As a brief summary of this study we report the foll0wings: a) Significant pathways identified by GeneGo in GSTT1*0 and XRCC1*4 wildtypes, are found to be predominantly part of the immune response. In these variants, a number of apoptosis and cell survival pathways suggest useful role in therapies of cancer and age related diseases. b) Concerning GSTT1*0 deletion variants, significant pathways are found to be related to G-protein signalling.

Additionally, a number of homeostatic pathways important in maintenance of tissues and in response to environmental cues are found which suggest useful role in prevention of cardiovascular and neurodegenerative diseases. c) With regards to XRCC1*4 heterozygous and homozygous variants, pathways modulations are found to be mainly related to glutathione metabolism. Blueberry intervention in these variants are shown to contribute to pathways involved in oxidative stress which suggest a role in (prevention) of age related diseases for subjects carrying XRCC1*4 heterozygous and homozygous variants. A visual illustration of study results are presented by means of figures 10 and 11.

Conclusions

By 2020, the world population is expected to have increased to 7.5 billion; of this number, approximately 15 million new cancer cases will be diagnosed, and 12 million cancer patients will die (4). The incidence of chronic diseases are rapidly increasing worldwide therefore it is important to identify dietary preventive measures or complementary approaches to existing medications.

The burden of modern lifestyle diseases is enormous as the true unifying factor for most cancers seems to be lifestyle. The intervention with the modification of lifestyle factors seems to be a physiological and safe approach for the prevention and management of modern lifestyle diseases including cancer. Defining the precise role of diet and other lifestyle factors, in the context of the pathogenesis of chronic disease, requires the elucidation of genetic susceptibility and genetic-environmental interactions.

Through this investigation of the whole genome gene expression changes in lymphocytes of healthy human subjects following a dietary blueberry intervention, in combination with specific polymorphism subgroups of GSTT1- and XRCC1-, we were able to identify differences in pathways and cellular processes modulated in each subgroup. This study reports newly identified genes which attenuate the role of specific signaling pathways that may play key roles in (reducing) the incidence of chronic diseases such as cardiovascular disease, neurodegenerative disease and cancer.

Further elucidation of mechanisms of action of fruits and vegetables at the cellular and molecular levels are essential in order to lay the foundation for the development of diet-based strategies for the prevention and therapy of several important types of chronic diseases. It is also important to emphasize that the study of the combined effect of SNPs in different genes, will be promising since gene-nutrient interactions are quite complex.

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References

1. Kushi LH, Byers T, Doyle C, Bandera EV, McCullough M, McTiernan A, Gansler T, Andrews KS, Thun MJ. American Cancer Society 2006 Nutrition and Physical Activity Guidelines Advisory Committee. American Cancer Society Guidelines on Nutrition and Physical Activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. CA Cancer J Clin. 2006;56(5):254–81.
2. World Cancer Research Fund/American Institute for Cancer Research. Food, Nutrition, and Physical Activity, and the Prevention of Cancer: A Global Perspective. Washington, DC: AICR; 2007.
3. Dietary fibre and colorectal cancer. N Engl J Med. 1999 Jun 17;340(24):1925; author reply 1926.
4. Predicting the future burden of cancer.Nat Rev Cancer. 2006 Jan;6(1):63-74.
5. Nutraceuticals as anti-angiogenic agents: hopes and reality. J Physiol Pharmacol. 2005 Mar;56 Suppl 1:51-67.
6. Stavric,B. (1994) Quercetin in our diet: from potent mutagen to probable anticarcinogen. Clin. Biochem., 27, 245–248.
7. Wattenberg,L.W. (1990) Inhibition of carcinogenesis by naturallyoccurring and synthetic compounds. Basic Life Sci., 52, 155–166.
8. Wattenberg,L.W. (1992) Inhibition of carcinogenesis by minor dietary onstituents. Cancer Res., 52, 2085s–2091s.
9. Prior, R. L., Cao, G., Martin, A., Sofic, E. et al., Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity and variety of Vaccinium species, J. Agric.
10. Food Chem. 1998, 46, 2686–2693 Fabiani, R., et al., Antioxidants prevent the lymphocyte DNA damage induced by PMA-stimulated monocytes. Nutr Cancer, 2001. 39(2): p. 284-91.
11. Noroozi, M., W.J. Angerson, and M.E. Lean, Effects of flavonoids and vitamin C on oxidative DNA damage to human lymphocytes. Am J Clin Nutr, 1998. 67(6): p. 1210-8.
12. Juranic, Z., Zizak, Z., Biological activities of berries: From antioxidant capacity to anti-cancer effects, Biofactors 2005, 23, 207–211.
13. Prior, R. L., Fruits and vegetables in the prevention of cellular oxidative damage, Am. J. Clin. Nutr. 2003, 78, 570S–578S.
14. Bub A, Watzl B, Blockhaus M, et al. Fruit juice consumption modulates antioxidative status, immune status and DNA damage. J Nutr Biochem 2003;14:90-8.
15. McAnulty SR, McAnulty LS, Morrow JD, et al. Effect of daily fruit ingestion on angiotensin converting enzyme activity, blood pressure, and oxidative stress in chronic smokers. Free Radic Res 2005;39:1241-8.
16. Wilms LC, Boots AW, de Boer VC, et al. Impact of multiple genetic polymorphisms on effects of a 4-week blueberry juice intervention on ex vivo induced lymphocytic DNA damage in human volunteers. Carcinogenesis 2007;28:1800-6.28.
17. Wilms LC, Hollman PC, Boots AW, Kleinjans JC. Protection by quercetin and quercetin-rich fruit juice against induction of oxidative DNA damage and formation of BPDE-DNA adducts in human lymphocytes. Mutat Res 2005;582:155-62.
18. Wilms L.C. et al(2007)Impact of multiple genetic polymorphisms on effects of a 4-week blueberry juiceintervention on ex vivo induced lymphocytic DNA damage in human volunteers. Carcinogenesis vol.28 no.8 pp.1800–1806, 2007.
19. Dusinská M, Ficek A, Horská A, Raslová K, Petrovská H, Vallová B, Drlicková M, Wood SG, Stupáková A, Gasparovic J, Bobek P, Nagyová A, Kováciková Z, Blazícek P, Liegebel U, Collins AR; Glutathione S-transferase polymorphisms influence the level of oxidative DNA damage and antioxidant protection in humans. Mutat Res. 2001 Oct 1;482(1-2):47-55.
20. Yuan LH, Meng LP, Ma WW, Li S, Feng JF, Yu HL, Xiao R; The role of glutathione S-transferase M1 and T1 gene polymorphisms and fruit and vegetable consumption in antioxidant parameters in healthy subjects. Br J Nutr. 2012 Mar;107(6):928-33. Epub 2011 Sep 7.
21. Capoluongo E, Almadori G, Concolino P, Bussu F, Santonocito C, Vendittelli F, Galli J, Zuppi C, Ameglio F, Paludetti G, Giardina B.; GSTT1 and GSTM1 allelic polymorphisms in head and neck cancer patients from Italian Lazio Region. Clin Chim Acta. 2007 Feb;376(1-2):174-8. Epub 2006 Aug 17.
22. Singh M, Shah PP, Singh AP, Ruwali M, Mathur N, Pant MC, Parmar D. Association of genetic polymorphisms in glutathione S-transferases and susceptibility to head and neck cancer. Mutat Res. 2008 Feb 1;638(1-2):184-94. Epub 2007 Oct 16.
23. Oude Ophuis MB, Manni JJ, Peters WH.Glutathione S-transferase T1 null polymorphism and the risk for head and neck cancer. Acta Otolaryngol. 2006 Mar;126(3):311-7.
24. Shu Zhang, Jixin Zhong, Ping Ya, Feili Gong, and Cong-Yi Wang; HMGB1, an innate alarmin, in the pathogenesis of type 1 diabetes ;Int J Clin Exp Pathol. 2010; 3(1): 24–38.
25. Anding Liu,Haoshu Fang, Olaf Dirsch, Hao Jin, and Uta Dahmen; Oxidation of HMGB1 Causes Attenuation of Its Pro-Inflammatory Activity and Occurs during Liver Ischemia and Reperfusion ; PLoS One. 2012; 7(4): e35379.
26. Mukhopadhyay S, Hoidal JR, Mukherjee TK ; Role of TNFalpha in pulmonary pathophysiology. Respiratory research 2006 Oct 11;7:125.
27. Virginie M Renoux,Marc Thiry, Philippe Delvenne and Nathalie Jacobs ; Natural killer cells: role in local tumor growth and metastasis; Inge Langers,; Biologics. 2012; 6: 73–82.
28. McAnulty LS, Nieman DC, Dumke CL, Shooter LA, Henson DA, Utter AC, Milne G, McAnulty SR; Effect of blueberry ingestion on natural killer cell counts, oxidative stress, and inflammation prior to and after 2.5 h of running;;Appl Physiol Nutr Metab. 2011 Dec;36(6):976-84. Epub 2011 Nov 23.
29. Turrin NP, Rivest S ;Unraveling the molecular details involved in the intimate link between the immune and neuroendocrine systems. Experimental biology and medicine (Maywood, N.J.) 2004 Nov;229(10):996-1006.
30. Platanias LC ;Mechanisms of type-I- and type-II-interferon-mediated signalling. Nature reviews. Immunology 2005 May;5(5):375-86.
31. Ramana CV, Gil MP, Schreiber RD, Stark GR ;Stat1-dependent and -independent pathways in IFN-gamma-dependent signaling. Trends in immunology 2002 Feb;23(2):96-101.
32. de Rooij J, Rehmann H, van Triest M, Cool RH, Wittinghofer A, Bos JL ;Mechanism of regulation of the Epac family of cAMP-dependent RapGEFs. The Journal of biological chemistry 2000 Jul 7;275(27):20829-36.
33. Hollinger S, Ramineni S, Hepler JR , Phosphorylation of RGS14 by protein kinase A potentiates its activity toward G alpha i. Biochemistry 2003 Jan 28;42(3):811-9.
34. Greco F, Sinigaglia F, Balduini C, Torti M ;Activation of the small GTPase Rap2B in agonist-stimulated human platelets. Journal of thrombosis and haemostasis : JTH 2004 Dec;2(12):2223-30.
35. Greco F, Ciana A, Pietra D, Balduini C, Minetti G, Torti M ;Rap2, but not Rap1 GTPase is expressed in human red blood cells and is involved in vesiculation. Biochimica et biophysica acta 2006 Mar;1763(3):330-5.
36. Rebhun JF, Castro AF, Quilliam LA ;Identification of guanine nucleotide exchange factors (GEFs) for the Rap1 GTPase. Regulation of MR-GEF by M-Ras-GTP interaction. The Journal of biological chemistry 2000 Nov 10;275(45):34901-8.

37. Stope MB, Vom Dorp F, Szatkowski D, Bohm A, Keiper M, Nolte J, Oude Weernink PA, Rosskopf D, Evellin S, Jakobs KH, Schmidt MRap2B-dependent stimulation of phospholipase C-epsilon by epidermal growth factor receptor mediated by c-Src phosphorylation of RasGRP3. Molecular and cellular biology 2004 Jun;24(11):4664-76.
38. Buchsbaum RJ ;Rho activation at a glance. Journal of cell science 2007 Apr 1;120(Pt 7):1149-52.
39. Sternweis PC, Carter AM, Chen Z, Danesh SM, Hsiung YF, Singer WD ; Regulation of Rho guanine nucleotide exchange factors by G proteins. Advances in protein chemistry 2007;74:189-228.
40. Ohba Y, Mochizuki N, Yamashita S, Chan AM, Schrader JW, Hattori S, Nagashima K, Matsuda M; Regulatory proteins of R-Ras, TC21/R-Ras2, and M-Ras/R-Ras3. The Journal of biological chemistry 2000 Jun 30;275(26):20020-6.
41. Guerrero C, Martin-Encabo S, Fernandez-Medarde A, Santos E ;C3G-mediated suppression of oncogene-induced focus formation in fibroblasts involves inhibition of ERK activation, cyclin A expression and alterations of anchorage-independent growth. Oncogene 2004 Jun 17;23(28):4885-93.
42. Wu M, Deng L, Zhu G, Li YP. Front Biosci. ; G Protein and its signaling pathway in bone development and disease;2010 Jun 1;15:957-85.
43. Ritzén A, Mathiesen JM, Thomsen C.Molecular pharmacology and therapeutic prospects of metabotropic glutamate receptor allosteric modulators. Basic Clin Pharmacol Toxicol. 2005 Oct;97(4):202-13.
44. Wang Q, Dziarski R, Kirschning CJ, Muzio M, Gupta D ; Micrococci and peptidoglycan activate TLR2-->MyD88-->IRAK-->TRAF-->NIK-->IKK-->NF-kappaB signal transduction pathway that induces transcription of interleukin-8. Infection and immunity 2001 Apr;69(4):2270-6.
45. Shi CS, Kehrl JH ;MyD88 and Trif target Beclin 1 to trigger autophagy in macrophages. The Journal of biological chemistry 2008 Nov 28;283(48):33175-82.
46. Vinay DS, Kwon BS ;Role of 4-1BB in immune responses. Seminars in immunology 1998 Dec;10(6):481-9.
47. Lee HW, Park SJ, Choi BK, Kim HH, Nam KO, Kwon BS ;4-1BB promotes the survival of CD8+ T lymphocytes by increasing expression of Bcl-xL and Bfl-1. Journal of immunology (Baltimore, Md. : 1950) 2002 Nov 1;169(9):4882-8.
48. Sabbagh L, Pulle G, Liu Y, Tsitsikov EN, Watts TH ;ERK-dependent Bim modulation downstream of the 4-1BB-TRAF1 signaling axis is a critical mediator of CD8 T cell survival in vivo. Journal of immunology (Baltimore, Md. : 1950) 2008 Jun 15;180(12):8093-101.
49. Palmer MJ, Mahajan VS, Trajman LC, Irvine DJ, Lauffenburger DA, Chen J ;Interleukin-7 receptor signaling network: an integrated systems perspective. Cellular and molecular immunology 2008 Apr;5(2):79-89.
50. Wofford JA, Wieman HL, Jacobs SR, Zhao Y, Rathmell JC ;IL-7 promotes Glut1 trafficking and glucose uptake via STAT5-mediated activation of Akt to support T-cell survival. Blood 2008 Feb 15;111(4):2101-11.
51. Kuo TC, Schlissel MS ;Mechanisms controlling expression of the RAG locus during lymphocyte development. Current opinion in immunology 2009 Apr;21(2):173-8.
52. Dickinson DA, Forman HJ ; Glutathione in defense and signaling: lessons from a small thiol. Annals of the New York Academy of Sciences 2002 Nov;973:488-504.
53. Lin MT, Beal MF;Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 2006 Oct 19;443(7113):787-95.
54. De Vega L, Perez Fernandez R, Martin Mateo MC, Bustamante J, Bustamante A, Herrero AM, Bustamante Munguira E ;Study of the activity of glutathione-peroxidase, glutathione-transferase, and glutathione-reductase in renal transplants. Transplantation proceedings 2003 Jun;35(4):1346-50.
55. Masoodi TA, Rao Talluri V, Shaik NA, Al-Aama JY, Hasan Q. Functional genomics based prioritization of potential nsSNPs in EPHX1, GSTT1, GSTM1 and GSTP1 genes for breast cancer susceptibility studies. Genomics. 2012 May 1.
56. Kiyohara C, Horiuchi T, Takayama K, Nakanishi Y; Genetic polymorphisms involved in carcinogen metabolism and DNA repair and lung cancer risk in a Japanese population. J Thorac Oncol. 2012 Jun;7(6):954-62.
57. Lee J. Martin, DNA Damage and Repair: Relevance to Mechanisms of Neurodegeneration; J Neuropathol Exp Neurol. 2008 May; 67(5): 377–387.
58. Caldecott KW; XRCC1 and DNA strand break repair. DNA Repair (Amst). 2003 Sep 18;2(9):955-69.
59. Sajeela Yousaf, Muhammad Imran Khan, Shazia Micheal, Farah Akhtar,Syeda Hafiza Benish Ali, Moeen Riaz, Mahmood Ali, Pramila Lall,Nadia Khalida Waheed, Anneke I. den Hollander, Asifa Ahmed, and Raheel Qamarcorresponding author XRCC1 and XPD DNA repair gene polymorphisms: A potential risk factor for glaucoma ; Mol Vis. 2011; 17: 1153–1163.
60. Sacca SC, Bolognesi C, Battistella A, Bagnis A, Izzotti A. Gene-environment interactions in ocular diseases. Mutat Res. 2009 Jul 10;667(1-2):98-117. Epub 2008 Nov 13.
61. Guoyao Wu2, Yun-Zhong Fang, Sheng Yang, Joanne R. Lupton, and Nancy D; TurnerGlutathione Metabolism and Its Implications for Health; J. Nutr. March 1, 2004 vol. 134 no. 3 489-492.
62. Anastasia Z. Kalea, Fotini N. Lamari, Achilleas D. Theocharis, Paul Cordopatis; Wild blueberry (Vaccinium angustifolium) consumption affects the composition and structure of glycosaminoglycans in Sprague-Dawley rat aorta; journal of of nutritional biochem(vol 17 , pg 109-116).
63. Oliveros JC. VENNY. An interactive tool for comparing lists with Venn Diagrams.; 2007. J. D. Hayes, M. O. Kelleher and I. M. Eggleston, Eur. J. Nutr., 2008,47(S2), 73–88.
64. J. D. Clarke, R. H. Dashwood and E. Ho, Cancer Lett., 2008, 269, 291–304.
65. S. E. Steck, M. M. Gaudet, J. A. Britton, S. L. Teitelbaum,M. B. Terry, A. I. Neugut, R. M. Santella and M. D. Gammon, Carcinogenesis, 2007, 28, 1954–1959.
66. H. Nian, B. Delage, E. Ho and R. H. Dashwood, Environ. Mol. Mutagen., 2009, 50, 213–221.
67. R. H. Dashwood and E. Ho, Semin. Cancer Biol., 2007, 17, 363–369.
68. J. W. Lampe and S. Peterson, J. Nutr., 2002, 132, 2991–2994.
69. D. J. Williams, C. Critchley, S. Pun, M. Chaliha and T. J. O'Hare, Phytochemistry, 2009, 70, 1401–1409).
70. Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galun E, Ben-Neriah Y. 2004. NF-κB functions as a tumour promoter in inflammation-associated cancer. Nature 431: 461–466.
71. Reuter, U., Chiarugi, A., Bolay, H., Moskowitz, M. A. Nuclear factor-kappa-B as a molecular target for migraine therapy. Ann. Neurol. 51: 507-516, 2002.
72. Bernard Thienpont, Jeroen Breckpot, Maureen Holvoet, Joris R. Vermeesch, Koen Devriendt; A microduplication of CBP in a patient with mental retardation and a congenital heart defec; American Journal of Medical Genetics Part A, Volume 143A, Issue 18, pages 2160–2164, 15 September 2007.
73. Bendjennat, M., J. Boulaire, T. Jascur, H. Brickner, V. Barbier, A. Sarasin, A. Fotedar, and R. Fotedar. 2003. UV irradiation triggers ubiquitin-dependent degradation of p21(WAF1) to promote DNA repair. Cell. 114:599–610.
74. Jonathan K. Alder, Nini Guo, Frant Kembou, Erin M. Parry1,Mary Armanios;Telomere Length Is a Determinant of Emphysema Susceptibility, Am. J. Respir. Crit. Care Med. October 15, 2011 vol. 184 no. 8 904-912.

75. Roger S.-Y. Foo, Lennard K. W. Chan, Richard N. Kitsis, and Martin R. Bennett; protein synthesis, post-translation modification, and degradation: ubiquitination and degradation of the anti-apoptotic protein arc by mdm2, J. Biol. Chem., Feb 2007; 282: 5529-5535.
76. A. Phillip West,Igor E. Brodsky,Christoph Rahner,Dong Kyun Woo; TLR signalling augments macrophage bactericidal activity through mitochondrial ROS; Nature 472, 476–480 (28 April 2011).
77. Myrto Poulou, Marios Kaliakatsos, Aspasia Tsezou, Emmanuel Kanavakis, Konstantinos N. Malizos, and Maria Tzetis. Genetic Testing. June 2008, 12(2): 263-265. doi:10.1089/gte.2007.0114.
78. Kabra, N.H., Kang, C., Hsing, L.C., Zhang, J., and Winoto, A. 2001. T cell-specific FADD-deficient mice: FADD is required for early T cell development. Proc. Natl. Acad. Sci. 98: 6307-6312.
79. Anne M. Landau,Kelvin C. Luk,Michelle-Lee Jones,et al, Defective Fas expression exacerbates neurotoxicity in a model of Parkinson's disease, JEM vol. 202 no. 5 575-581.
80. Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature. 2001;411:957–962.
81. Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature. 2001;411:957–962.
82. Antoniou, et al. (2011) Common alleles at 6q25.1 and 1p11.2 are associated with breast cancer risk for BRCA1 and BRCA2 mutation carriers. Human Molecular Genetics 2011 May 18.
83. Alberto Fernández-Medarde, Eugenio Santos; Ras in Cancer and Developmental Diseases, Genes & Cancer March 2011 vol. 2 no. 3 344-358.
84. Cynthia J. Kenyon; The genetics of ageing, Nature 464, 504–512 (25 March 2010).
85. Andrey S. Shaw & Erin L. Filbert; Scaffold proteins and immune-cell signalling, Nature Reviews Immunology 9, 47-56 (January 2009).
86. Tauzin S, Ding H, Burdevet D, Borisch B, Hoessli DC.; Membrane-associated signaling in human B-lymphoma lines., Exp Cell Res. 2011 Jan 15;317(2):151-62.
87. Dumka et al., Activation of the p38 Map kinase pathway is essential for the antileukemic effects of dasatinib. (2009).
88. Vinayagam A, Stelzl U, Foulle R, Plassmann S, Zenkner M, Timm J, Assmus HE, Andrade-Navarro MA, Wanker EE.; A directed protein interaction network for investigating intracellular signal transduction., Sci Signal. 2011 Sep 6;4(189):rs8.
89. Thomas K Schlesinger et al. (2002). "Apoptosis stimulated by the 91-kDa caspase cleavage MEKK1 fragment requires translocation to soluble cellular compartments".
90. Wong VW, Rustad KC, Akaishi S, Sorkin M, Glotzbach JP, Januszyk M,, Levi K, Paterno J, Vial IN, Kuang AA, Longaker MT, Gurtner GC. Nat Med. 2012 Jan; 18(1):148-52.
91. Claire J. Fearnley, H. Llewelyn Roderick and Martin D. Bootman; Calcium Signaling in Cardiac Myocytes; Cold Spring Harbor Laboratory Press; August 29, 2011.
92. Lejin Wang,et al. Mutation of MEF2A in an Inherited Disorder with Features of Coronary Artery Disease, Science 28 November 2003: Vol. 302 no. 5650 pp. 1578-1581.
93. Alessandro Rimessi, Rosario Rizzuto, and Paolo Pinton; Differential recruitment of PKC isoforms in HeLa cells during redox stress, Cell Stress Chaperones. 2007 December; 12(4): 291–298.
94. Yue Zhang, Yelena M. Altshuller, Scott M. Hammond, Andrew J. Morris, and Michael A. Frohman; Loss of receptor regulation by a phospholipase D1 mutant unresponsive to protein kinase C, The EMBO Journal (1999) 18, 6339-6348.
95. Craig W Lindsley and H Alex Brown; Phospholipase D as a Therapeutic Target in Brain Disorders, Neuropsychopharmacology 37, 301-302 (January 2012).
96. Laura Pasqualucci et al. Inactivating mutations of acetyltransferase genes in B-cell lymphoma; Nature 471, 189–195 (10 March 2011).
97. Mullighan CG, Zhang J, Kasper LH, Lerach S, Payne-Turner D, Phillips LA, Heatley SL, Holmfeldt L, Collins-Underwood JR, Ma J, et al. 2011. CREBBP mutations in relapsed acute lymphoblastic leukaemia. Nature 471: 235–239.
98. Vaira, S., Johnson, T., Hirbe, A.C., Alhawagri, M., Anwisye, I., Sammut, B., O'Neal, J., Zou, W., Weilbaecher, K.N., Faccio, R., and Novack, D.V.. RelB is the NF-kB subunit downstream of NIK responsible for osteoclast differentiation. Proc Natl Acad Sci 105:3897-3902, 2008.
99. Barbara Marinari et al.,The activation of Csk by CD4 interferes with TCR-mediated activatory signaling, European Journal of Immunology Volume 33, Issue 9, pages 2609–2618, September 2003.
100. Hammond SM, Altshuller YM, Sung T-C, Rudge SA, Rose K, Engebrecht J, Morris AJ and Frohman MA (1995) Molecular characterization of a human ARF-activated phosphatidylcholine-specific phospholipase D defines a new and highly conserved family of genes. J Biol Chem, 270, 29640–29643.
101. Landau et al. ,Defective Fas expression exacerbates neurotoxicity in a model of Parkinson's disease, Published August 29, 2005 // JEM vol. 202 no. 5 575-581.
102. All-Ericsson C,Insulin-like growth factor-1 receptor in uveal melanoma: a predictor for metastatic disease and a potential therapeutic target.; Invest Ophthalmol Vis Sci. 2002 Jan;43(1):1-8.

Figures

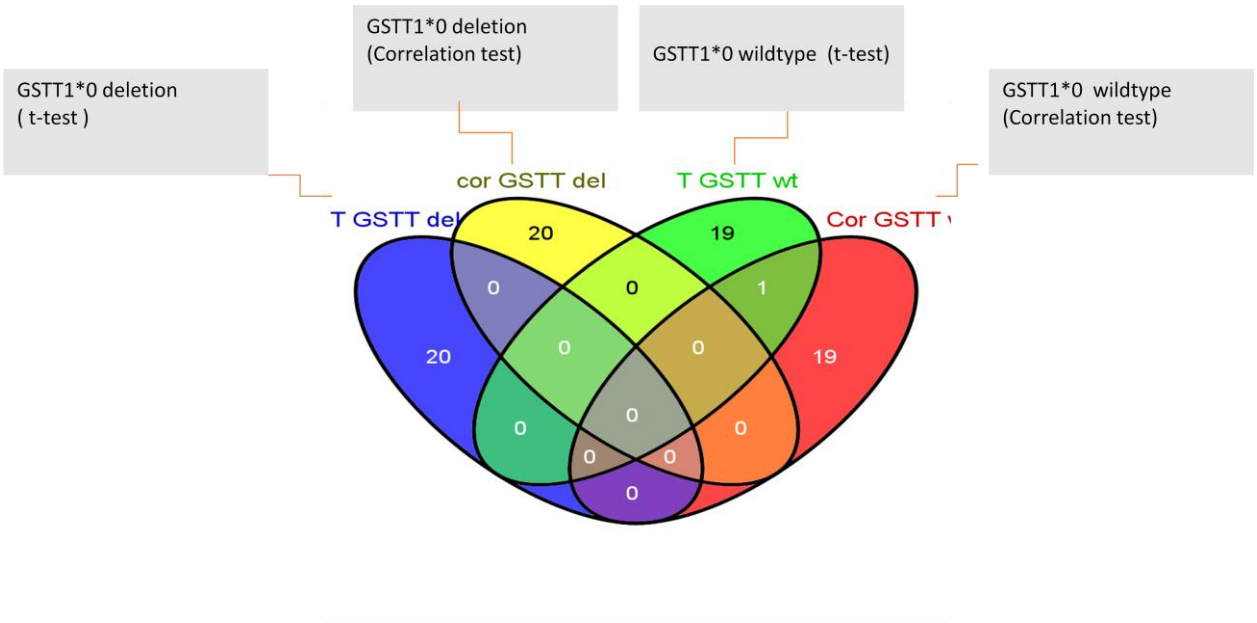

Figure 1. Venn diagram created using venny (63) of top twenty GeneGo biological pathways after pairwise t-test and correlation analysis, as found by Metacore™ Analysis of significantly modulated pathways for GSTT1*0 deletion and wildtype variants.

As depicted in this figure , comparison of the results between the paired t-test and correlation analysis showed no common elements at biological pathway levels for the GSTT1*0 deletion and wildtype variants. One (1) common pathway between the paired t-test analysis and correlation analysis of GSTT1*0 wildtype is identified which is identified as the "cytoskeleton remodeling- Alpha-1A adrenergic receptor-dependent inhibition of PI3K cellular process".

Numbers in each of the blue, yellow, green and red ovals refer to total quantity (20) of significant biological pathways that were compared using venny. Common quantity of pathways are shown in the joint areas between ovals.

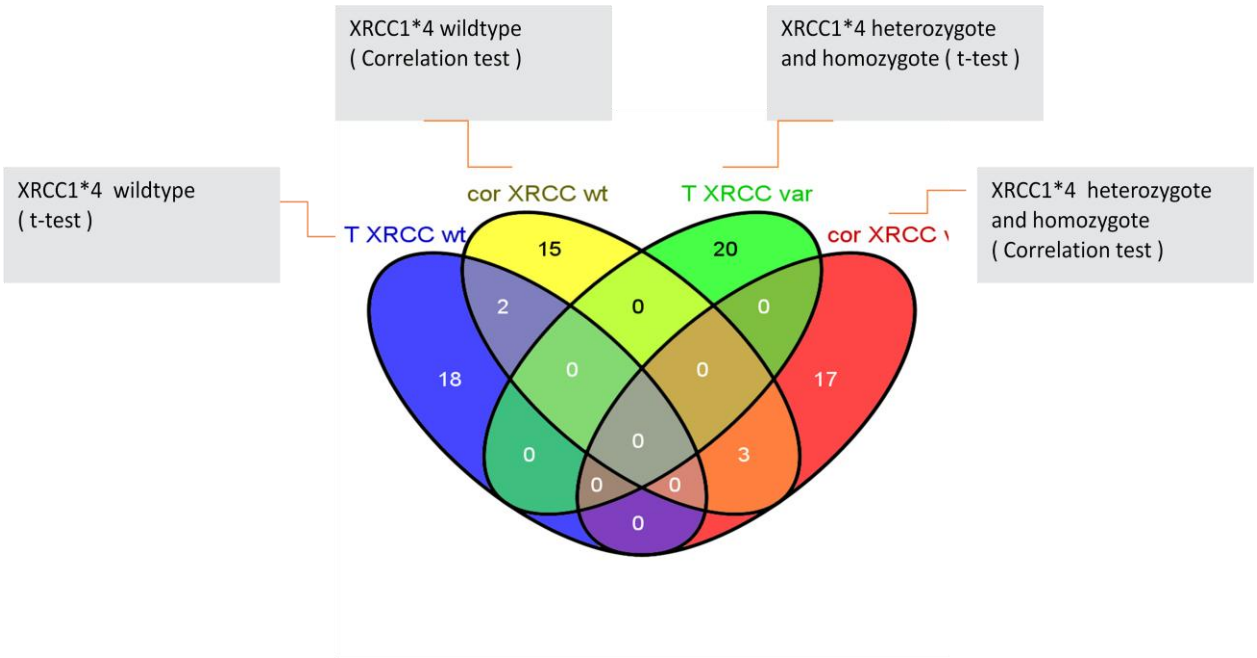

Figure 2- Venn diagram created using venny (63) of top twenty GeneGo biological pathways after pairwise t-test and correlation analysis, as found by Metacore^{TM} Analysis of significantly modulated pathways for XRCC1*4 wildtype and homozygous and heterozygous variants.

As depicted in this figure , comparison of the results between the paired t-test and correlation analysis showed no common elements at biological pathway levels for the XRCC1*4 wildtype and heterozygote and homozygote variants. Three (3)common pathways between the correlation analysis for XRCC1*4 wildtype and heterozygote and homozygote variants are identified as "Glutathione metabolism"; "Glutathione metabolism / Human version" ; and "Glutathione metabolism / Rodent version".

Additionally two(2) common pathways between the paired t-test and correlation analysis for XRCC1*4 wildtype are identified which refer to "Cytokine production by Th17 cells in CF (Mouse model)"; and "Nicotine signaling in dopaminergic neurons, Pt. 2 - axon terminal".

Numbers in each of the blue, yellow, green and red ovals refer to total quantity (20) of significant biological pathways that were compared using venny. Common quantity of pathways are shown in the joint areas between ovals.

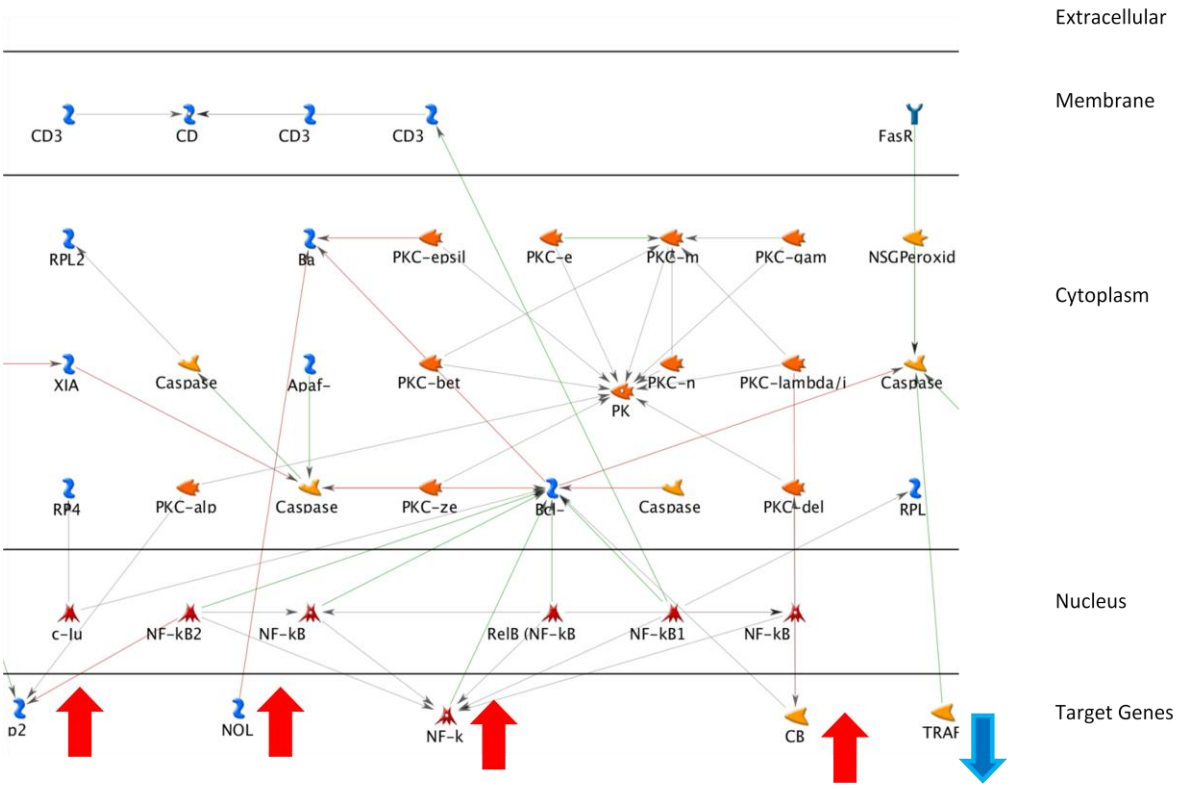

Figure 3- Shortest paths network pre-filtered on a sub-cellular level indicating the localization of gene expression of a selection of genes modulated in GSTT1*0 wildtype immune response pathways developed in Metacore™.

The genes for which the gene product eventually will result in a cellular effect were defined as "target genes" and grouped at the bottom of the figure.

The target genes identified in this figure were all up-regulated with the exception of TRAF-6 which was down -regulated by the 4-week blueberry intervention. The network of genes including p21, CBP, TRAF6, RelB (NF-kB subunit) and NOL3 are involved in regulation of cell death and apoptosis. Gene up-regulation is visualized by a **red color** arrow while down-regulation is visualized by a **blue color** arrow.

As identified by GeneGo analysis, the cellular processes in this network are involved in regulation of cell death (72.5%; 3.508e-24), regulation of apoptotic process (70.0%; 3.630e-23), regulation of programmed cell death (70.0%; 4.513e-23), induction of apoptosis (50.0%; 2.006e-22) and induction of programmed cell death (50.0%; 2.335e-22). The values in the brackets stated here indicate the percent of involvement in the GeneGo processes followed by the related p-value.

Abbreviations: Detailed information on the symbols can be found at http://www.genego.com/pdf/MC_legend.pdf.

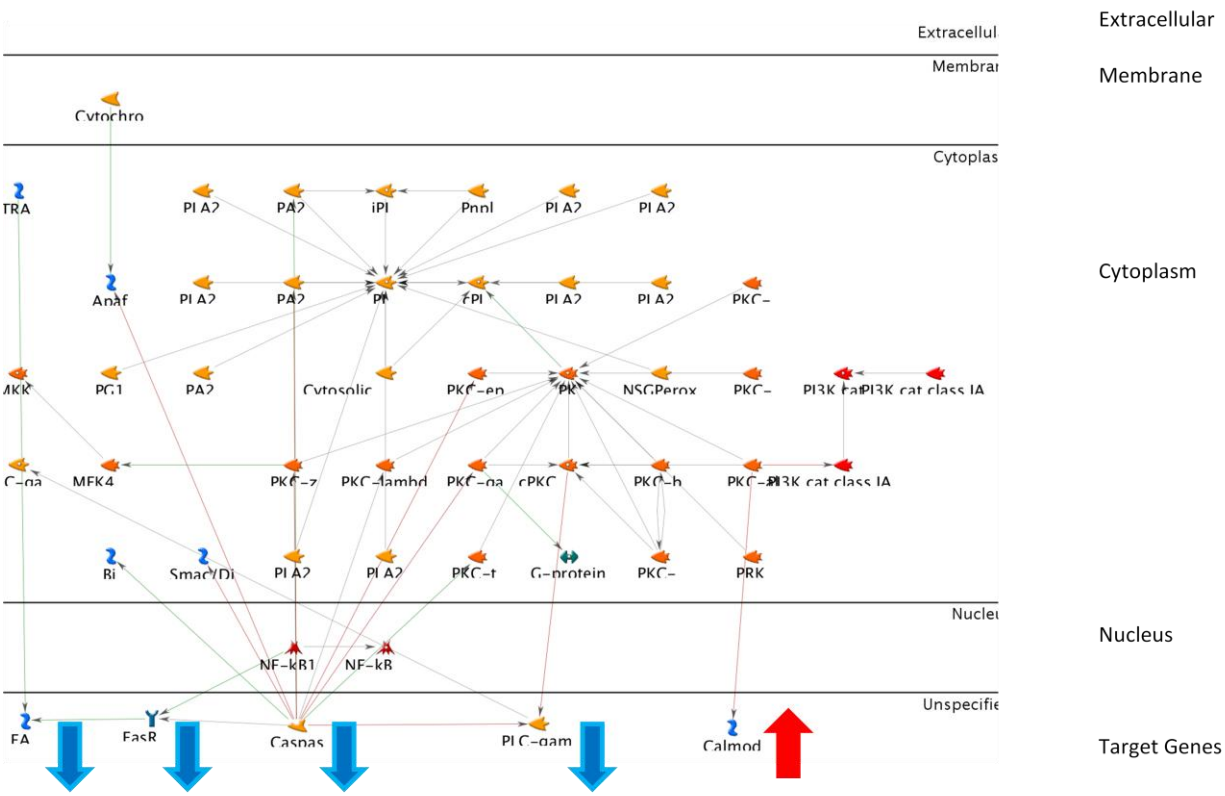

Figure 4- Shortest paths network pre-filtered on a sub-cellular level indicating the localization of gene expression of a selection of genes modulated in GSTT1*0 wildtype immune response pathways developed in Metacore™.

The genes for which the gene product eventually will result in a cellular effect were defined as target genes and grouped at the bottom of the figure.

The target genes identified at the bottom of this figure were all down-regulated with the exception of calmudulin which was up-regulated by the 4-week blueberry intervention. The network of genes including Calmodulin, Caspase-3, FasR(CD95), PLC-gamma 1 and FADD are involved in lipid catabolic process, phospholipid metabolic process, organophosphate metabolic process, positive regulation of molecular function and platelet activation. Gene up-regulation is visualized by a **red color** arrow while down-regulation is visualized by a **blue color** arrow.

As identified by GeneGo analysis, the cellular processes in this network are involved in lipid catabolic process (40.8%; 7.416e-26), phospholipid metabolic process (40.8%; 6.174e-25), organophosphate metabolic process (40.8%; 2.550e-24), positive regulation of molecular function (63.3%; 3.246e-23) and platelet activation (36.7%; 2.697e-20). The values in the brackets stated here indicate the percent of involvement in the GeneGo processes followed by the related p-value.

Abbreviations: Detailed information on the symbols can be found at http://www.genego.com/pdf/MC_legend.pdf.

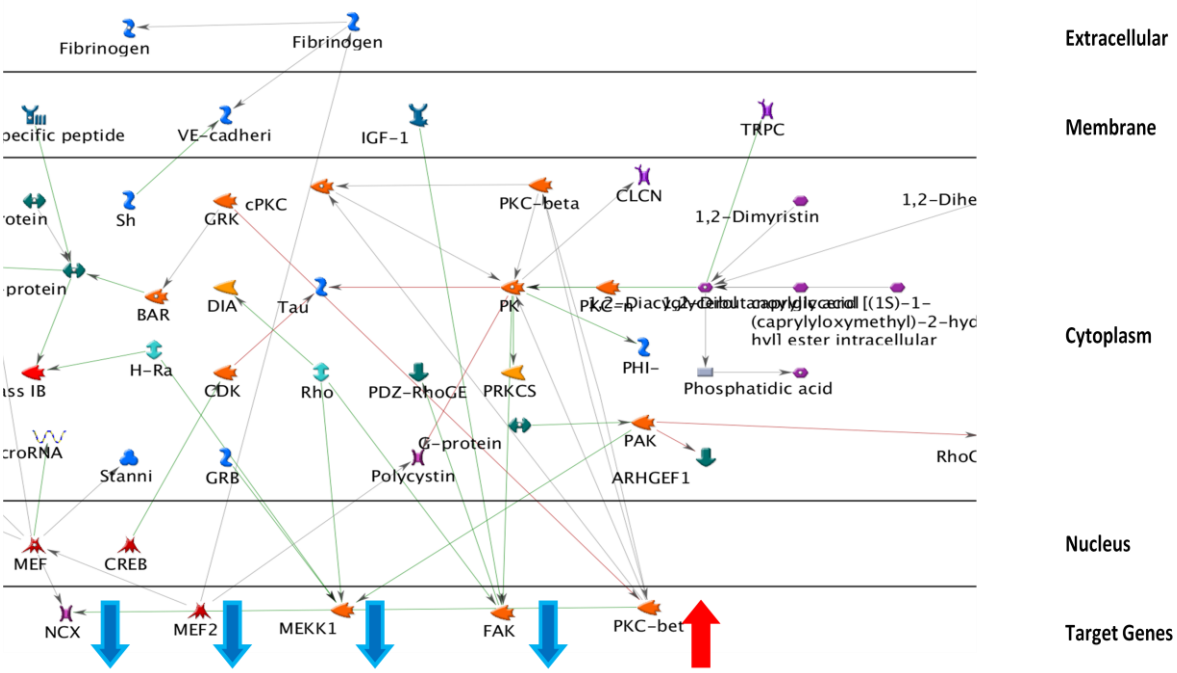

Figure 5- Shortest paths network pre-filtered on a sub-cellular level indicating the localization of gene expression of a selection of genes modulated in GSTT1*0 deletion G-protein signaling pathways developed in Metacore™.

The genes for which the gene product eventually will result in a cellular effect were defined as target genes and grouped at the bottom of the figure.

The target genes identified at the bottom of this figure were all down-regulated with the exception of PKC-beta which was up-regulated by the 4-week blueberry intervention. The network of genes including MEKK1 (MAP3K1), FAK1, NCX1, MEF2A and PKC-beta are involved in intracellular signal transduction, wound healing, platelet activation, regulation of body fluid levels and response to external stimulus. Gene up-regulation is visualized by a **red color** arrow while down-regulation is visualized by a **blue color** arrow.

As identified by GeneGo analysis, the cellular processes in this network are involved in intracellular signal transduction (61.4%; 4.268e-19), wound healing (47.7%; 5.140e-19), platelet activation (36.4%; 4.274e-18), regulation of body fluid levels (45.5%; 7.842e-18), and response to external stimulus (56.8%; 1.446e-17). The values in the brackets stated here indicate the percent of involvement in the GeneGo processes followed by the related p-value.

Abbreviations: Detailed information on the symbols can be found at http://www.genego.com/pdf/MC_legend.pdf

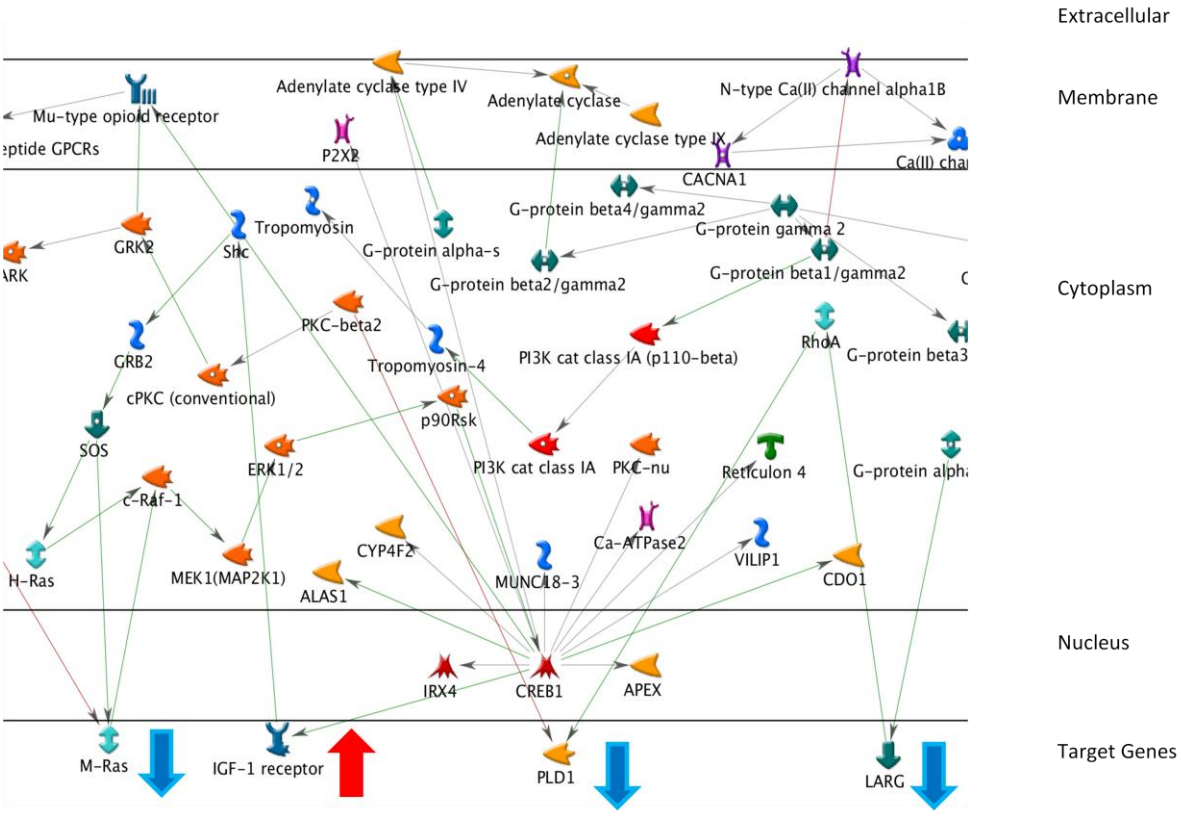

Figure 6-Shortest paths network pre-filtered on a sub-cellular level indicating the localization of gene expression of a selection of genes modulated in GSTT1*0 deletion G-protein signaling pathways developed in Metacore™.

The genes for which the gene product eventually will result in a cellular effect were defined as target genes and grouped at the bottom of the network.

The target genes identified at the bottom of this figure were all down-regulated with the exception of IGF-1 which was up-regulated by the 4-week blueberry intervention. The network of genes including M-Ras, PLD1, LARG, IGF-1 receptor and RASA2 are involved in response to peptide hormone and endogenous stimulus, in response to organic substance and in nerve growth factor receptor signaling pathway. Gene up-regulation is visualized by a **red color** arrow while down-regulation is visualized by a **blue color** arrow.

As identified by GeneGo analysis, the cellular processes in this network are involved in response to peptide hormone stimulus (56.0%; 1.366e-30), response to endogenous stimulus (70.0%; 1.411e-29), response to hormone stimulus (64.0%; 4.296e-28), response to organic substance (78.0%; 9.738e-27) and nerve growth factor receptor signaling pathway. The values in the brackets stated here indicate the percent of involvement in the GeneGo processes followed by the related p-value.

Abbreviations: Detailed information on the symbols can be found at http://www.genego.com/pdf/MC_legend.pdf.

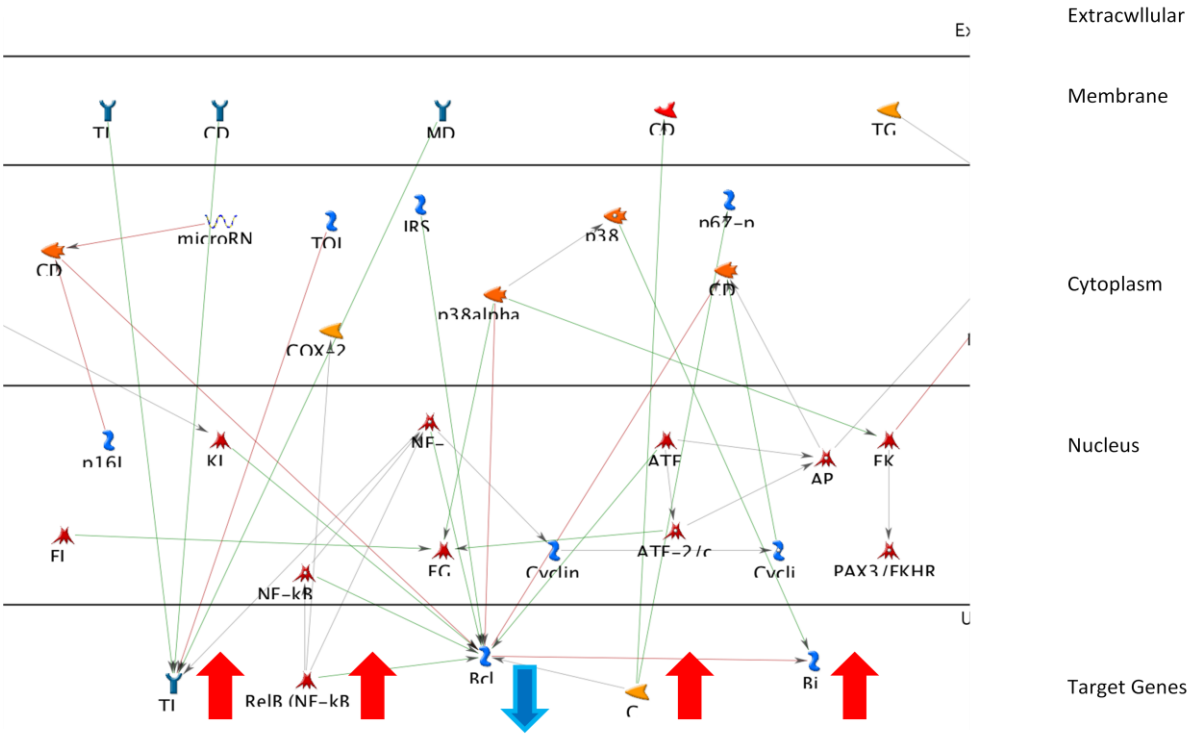

Figure 7-Shortest paths network pre-filtered on a sub-cellular level indicating the localization of gene expression of a selection of genes modulated in XRCC1*4 wildtypes in immune response pathways developed in Metacore™.

The genes for which the gene product eventually will result in a cellular effect were defined as target genes and grouped at the bottom of the figure.

The target genes identified at the bottom of this figure were all upregulated, with the exception of BCL-2 which was down-regulated by the 4-week blueberry intervention. The network of genes including Bcl-2, TLR4, CBP, RelB (NF-kB subunit) and Bim, involved in positive regulation of metabolic process, positive regulation of biological process and innate immune response. Gene up-regulation is visualized by a **red color** arrow while down-regulation is visualized by a **blue color** arrow.

As identified by GeneGo analysis, the cellular processes in this network are involved in positive regulation of metabolic process (77.1%; 1.261e-20), positive regulation of macromolecule metabolic process (74.3%; 2.361e-20), positive regulation of biological process (88.6%; 4.512e-19), positive regulation of cellular metabolic process (68.6%; 4.370e-17), and innate immune response (45.7%; 2.392e-16). The values in the brackets stated here indicate the percent of involvement in the GeneGo processes followed by the related p-value.

Abbreviations: Detailed information on the symbols can be found at http://www.genego.com/pdf/MC_legend.pdf.

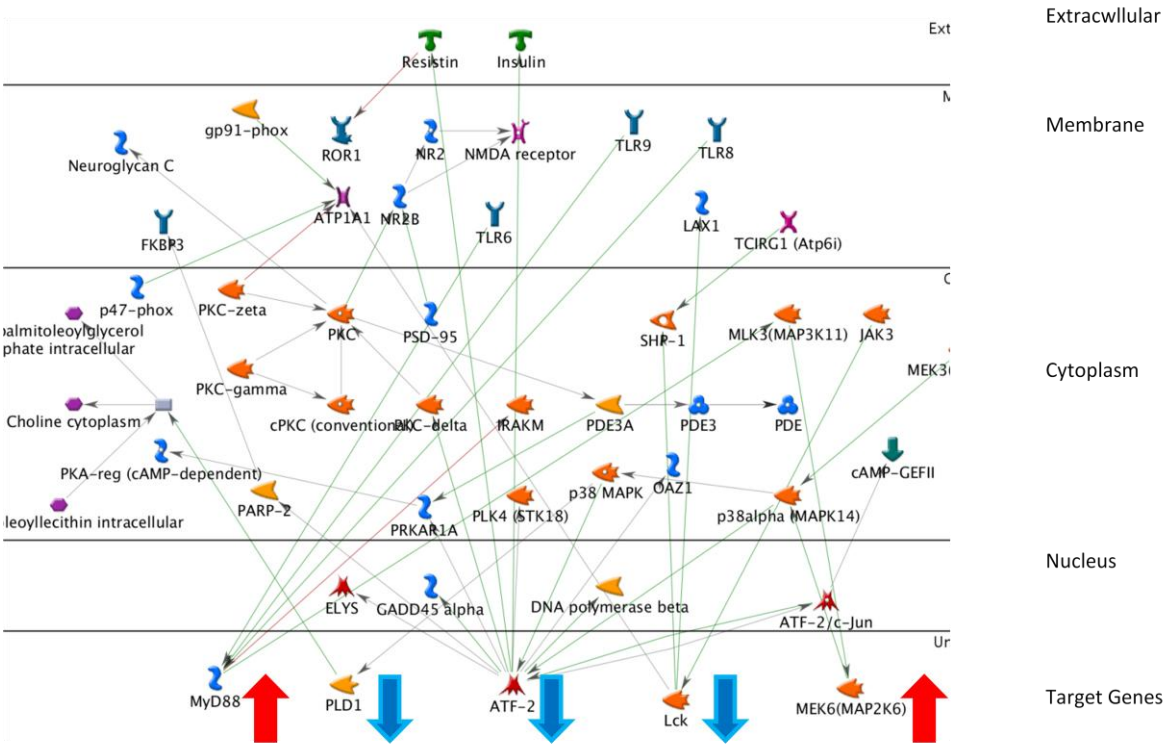

Figure 8-Shortest paths network pre-filtered on a sub-cellular level indicating the localization of gene expression of a selection of genes modulated in XRCC1*4 wildtypes in immune response pathways developed in Metacore™.

The genes for which the gene product eventually will result in a cellular effect were defined as target genes and grouped at the bottom of the network.

The target genes identified at the bottom of this figure were all down-regulated with the exception of MYD88 and MEK6 which were up-regulated by the 4-week blueberry intervention. The network of genes including ATF-2, MyD88, Lck, MEK6 (MAP2K6) and PLD1 is involved in response to stress, response to wounding, immune response-activating signal transduction, immune response-regulating signaling pathway and in regulation of protein metabolic process. Gene up-regulation is visualized by a **red color** arrow while down-regulation is visualized by a **blue color** arrow.

As identified by GeneGo analysis, the cellular processes in this network are involved in response to stress (80.9%; 9.812e-23), response to wounding (57.4%; 3.073e-21), immune response-activating signal transduction (34.0%; 2.250e-19), immune response-regulating signaling pathway (34.0%; 5.298e-19), regulation of protein metabolic process (57.4%; 3.477e-18). The values in the brackets stated here indicate the percent of involvement in the GeneGo processes followed by the related p-value.

Abbreviations: Detailed information on the symbols can be found at http://www.genego.com/pdf/MC_legend.pdf.

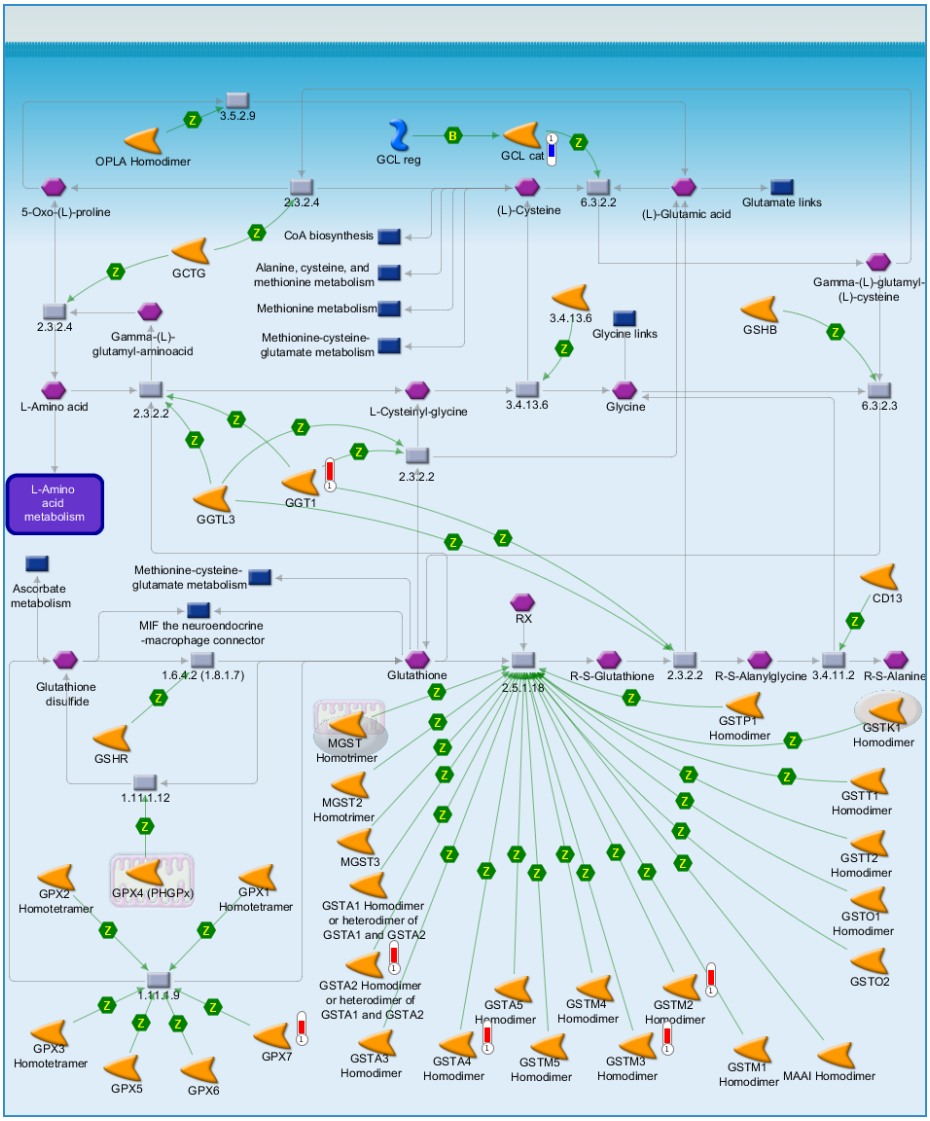

Figure 9 - Glutathione metabolism pathway- XRCC1*4 Heterzygote and Homozygote Variants.

This pathway with significant ranking is selected for its role in immune response pathways. Ranking was done using significant gene list for XRCC1*4 heterozygote and homozygote variants compared against the background list of genes for all study subjects.

The rankings are visualized using GeneGo ontology by Metacore™. As we can see from this picture, a few genes involved in glutathione metabolism, were significantly modulated : GGT1, GSTM2 homodimer, GSTM3, GSTA4, GPX7 and GSTA2 homodimer, were all up -regulated while GCL cat, was down-regulated..Gene up-regulation (equal or > 1) is visualized by a **dark red** while down-regulation (≤1) is visualized by a **dark blue** color.

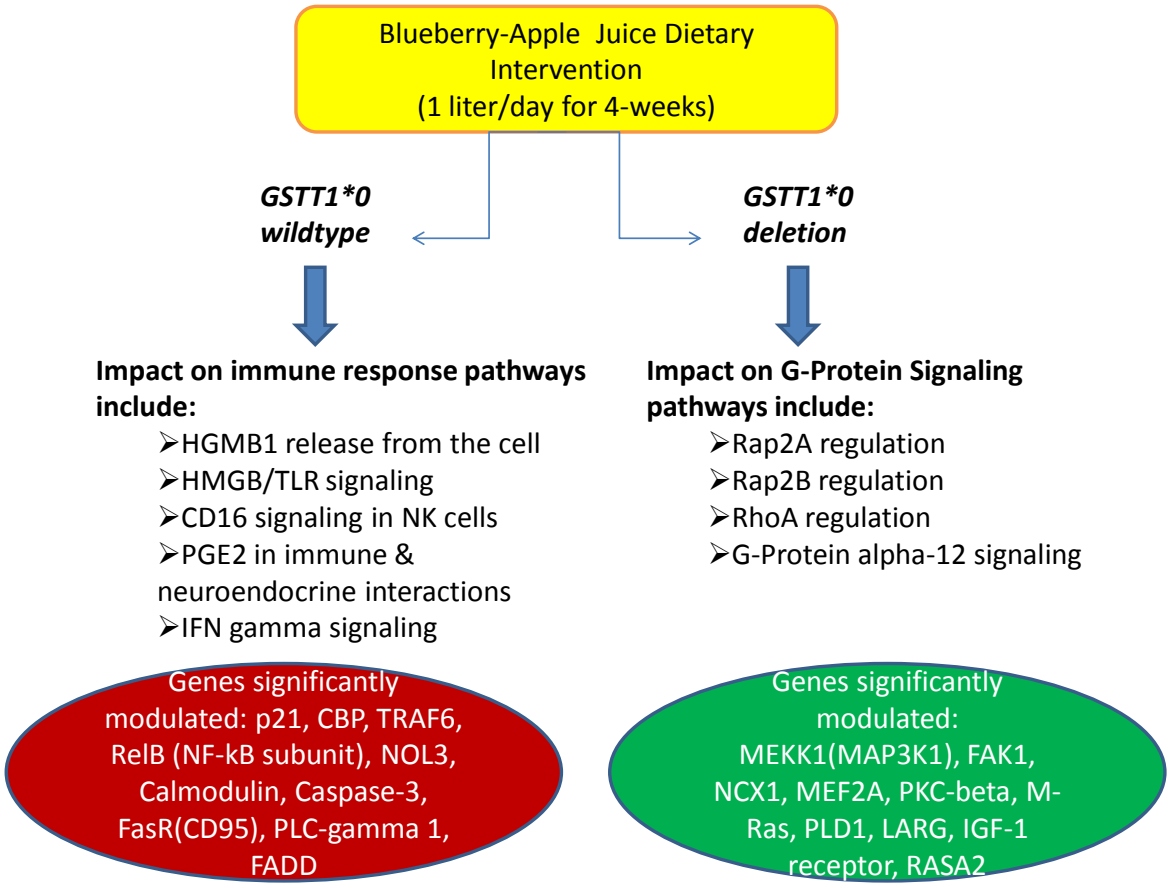

Figure 10 - Summary of Study Results for GSTT1*0 Wildtype and Deletion Variants

MetaCore GeneGo analysis of pathways and cellular processes for subjects following the 4-week blueberry intervention, identified some differences in pathways that are significantly ($p < 0.05$) modulated in the GSTT1*0 wildtype and null genotypes. In case of GSTT1*0 wt variants, the significant pathways are mainly part of the "immune response" cellular processes while for the GSTT1*0 deletion variants, significant pathways are part of the "G-protein signalling" cellular process.

Overall, for GSTT1*0 wildtypes, an up-regulation of CREB binding protein (CBP), Cyclin-dependent kinase inhibitor 1A (p21, Cip1), nucleolar protein 3 (NOL3), TNF receptor-associated factor 6 (TRAF6), apoptosis-related cysteine peptidase (Caspase 3), Calmodulin (CALM1) have been observed. While a down-regulation of Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1(NF-*k*B), (TNFRSF6)-associated via death domain(FADD), TNF receptor super family, member 6 (FAS R), Phospholipase C and gamma 1 (PLC gamma) were observed.

In case of GSTT1*0 null variants, overall, an up-regulation of Phospholipase D1, phosphatidylcholine-specific(PLD1) and Insulin-like growth factor 1 receptor (IGF-1 receptor) have been observed. While a down-regulation of Mitogen-activated protein kinase 1 MEKK1(MAP3 K1), PTK2 protein tyrosine kinase 2 (FAK1), Solute carrier family 8 (sodium/calcium exchanger), member 1(NCX1), Myocyte enhancer factor 2A (MEF2A), Protein kinase C, beta 1 (PKC-beta1), Muscle RAS oncogene homolog (M-Ras), Rho guanine nucleotide exchange factor (GEF) 12 (LARG) and RAS p21 protein activator 2 (RASA2) were observed.

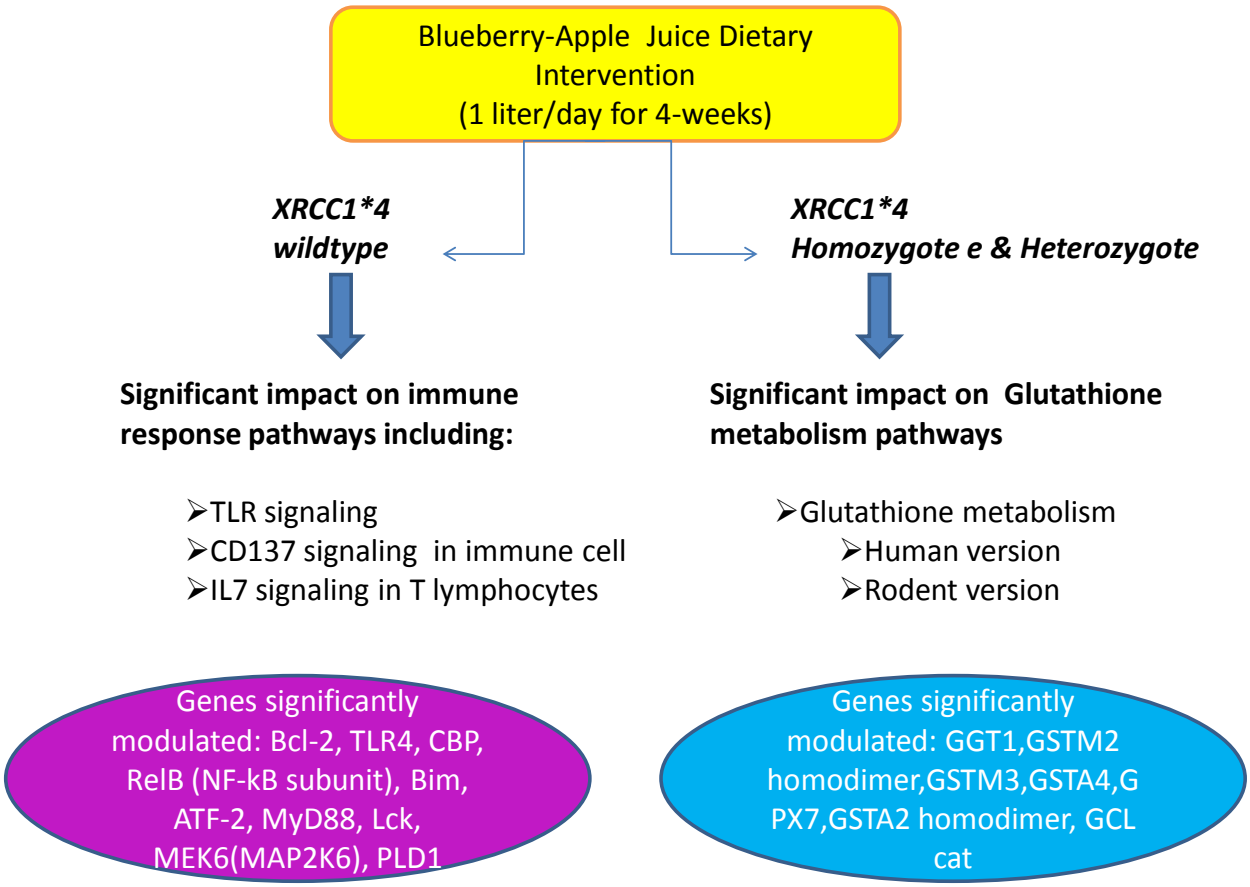

Figure 11-Summary of Study Results for XRCC1*4 Wildtype , Heterozygote and Homozygote Variants.

MetaCoreTM GeneGo analysis of pathways and cellular processes for subjects following the 4-week blueberry intervention identified differences in pathways are significantly ($p < 0.05$) modulated for XRCC1*4 wild type and XRCC1*4 heterozygous and homozygous variants.
As for the XRCC1*4 wild type, the significant pathways modulated are mostly related to the immune response cellular processes while the XRCC1*4 heterozygous and homozygous variants involvement is mainly in glutathione metabolism cellular processes.
In case of XRCC1*4 wild types, overall, an up regulation of Toll-like receptor 4(TLR4), BCL2-like 11 (apoptosis activator)(Bim), V-rel reticuloendotheliosis viral oncogene homolog B RelB (NF-kB subunit), CREB binding protein (CBP), Myeloid differentiation primary response gene (88) (MyD88), and Mitogen-activated protein kinase kinase 6-MEK6(MAP2K6) have been observed. While a down regulation of BCL2-like 11 (apoptosis facilitator) (Bcl-2), Activating transcription factor 2 (ATF-2), Lymphocyte-specific protein tyrosine kinase (LCK), Phospholipase D1, and phosphatidylcholine-specific (PLD1) were observed in this study.
With regards to the XRCC1*4 wt heterozygote and homozygote variants, genes involved in glutathione metabolism, were modulated. Study results show that GGT1, GSTM2 homodimer, GSTM3, GSTA4, GPX7 and GSTA2 homodimer, were all up -regulated while GCL cat, was down-regulated.

Supplementary Tables

Table 1. Single Nucleotide Polymorphisms

Single Nucleotide Polymorphism	Amino acid change	dbSNP ID	Function	Effect on enzymatic function	Frequencies wt / hz / v*
GSTT1*0	deletion		Phase II detoxification	no enzymatic activity	139/--/29
XRCC1*4	Q399R	rs25487	DNA repair	decreased enzyme activity	66/77/25

Study subjects were genotyped for a total of 34 single nucleotide polymorphisms (SNPs). The SNPs were selected based on a known or an expected association with oxidative stress, biotransformation of quercetin and B[a]P, and DNA repair. DNA for genotyping was isolated from lymphocytes by standard phenol extraction procedures.
This table lists the analyzed SNPs , universal ID codes, the amino acid change related to the GSTT1* - and XRCC1*-polymorphism , enzyme function and the expected effect of the polymorphism. The last column of this table represents the frequency of wild types, heterozygous and variants of these polymorphisms in the study population. The Cancer SNP 500 database was used to obtain DNA sequences and allele frequencies (http://snp500cancer.nci.nih.gov).

*: wt = homozygous wildtype, hz = heterozygous, v = homozygous variant.
Numbers in the far right column reflect the number of subjects carrying that genotype; a hyphen indicates that the method was not able to distinguish between heterozygous or homozygous wild type (in case of a deletion), therefore both of these polymorphisms are gathered under homozygous wild type.

Table 2- Polymorphism Subgroups Defined by Gene Expression Analysis (N=147)

Polymorphisms Subgroup	Variant	N	Significantly Modulated Subgroup Specific Genes[#]
GSTT1*0	**Wildtype**	122	**5765**
	Homozygous	25	2179
XRCC1*4	**Wildtype**	57	**1410**
	Heterozygous and homozygous	91	6274

Gene expression analysis of study subjects (n=147), identified 5765 and 1410 significantly ($p < 0.05$) modulated genes after the 4-week blueberry intervention, for GSTT1*0 and XRCC1*4 wildtypes, respectively.

Includes annotated and non-annotated genes. Significantly differentially expressed gene lists after the intervention were generated using ArrayTrackTM by means of pair wise t-tests ($p < 0.05$). Venn diagrams were created in ArrayTrackTM in which the significantly differentially expressed gene lists were incorporated for each group, thereby showing the number of genes which were specifically modulated in each subgroup separately.

Table 3. Metacore™ Analysis of GSTT1*0 Wildtype and Deletion Variants Following T-Test Assay: Genego Ranking of Cellular Processes and Biological Pathways Significantly Modulated in Lymphocytes of Study Subjects Consuming 1 Litre of Blueberry-Apple Juice Daily for 4 Weeks

Cellular Process	Pathway Involved	Number of Significant Genes per Group#		**Total No of Genes in Pathway**
		GSTT1*0 wildtype (n=122)	GSTT1*0 deletion (n=25)	
Development	Transcription regulation of granulocyte development		16	28
	PDGF signaling via STATs and NF-*k*B		11	19
	Angiotensin signaling via STATs		9	15
	ERK5 in cell proliferation and neuronal survival	8		21
	Growth hormone signaling via PI3K/AKT and MAPK cascades	10		36
	GH-RH signaling	5		12
	WNT signaling pathway. Part 1. Degradation of beta-catenin in the absence WNT signaling	5		12
	PEDF signaling	8		28
	Endothelin-1/EDNRA transactivation of EGFR	7		23
	Role of HDAC and calcium/calmodulin-dependent kinase (CaMK) in control of skeletal myogenesis	9		36
Immune Response	Th1 and Th2 cell differentiation		14	26
	IL-7 signaling in T lymphocytes		13	24
	Antiviral actions of Interferons		13	24
	Immune response_IL-23 signaling pathway		8	12
	Antigen presentation by MHC class I		10	17
	Immune response_CD28 signaling		15	30
	Immune response_NFAT in immune response		13	25
	Immune response _CCR3 signaling in eosinophils		18	39
	ICOS pathway in T-helper cell		13	26

	Classical complement pathway		13	27
	IFN alpha/beta signaling pathway		10	19
	IL-15 signaling via JAK-STAT cascade		10	20
	IL-7 signaling in B lymphocytes		14	30
	NF-AT signaling and leukocyte interactions		10	20
	IL-13 signaling via JAK-STAT		12	26
	Function of MEF2 in T lymphocytes	9		32
	Gastrin in inflammatory response	11		44
	IL-3 activation and signaling pathway	7		22
	CD137 signaling in immune cell	7		24
	Lipoxins and Resolvin E1 inhibitory action on neutrophil functions	5		14
	MIF-mediated glucocorticoid regulation	5		15
Apoptosis and survival	nAChR in apoptosis inhibition and cell cycle progression		9	16
	Beta-2 adrenergic receptor anti-apoptotic action		6	10

Paired t-test analysis, comparing gene expression profiles before and after the blueberry intervention for each subject, was performed in study population in order to investigate the gene expression effect in specific genetic subgroups which benefit more from the intervention as compared to their genetic counterparts. For GSTT1*0, subjects carrying the wildtype variant showed an overall significant decrease in oxidative DNA damage in ex vivo exposed lymphocytes after the 4-week blueberry intervention.

Analysis by Metacore^{TM} GeneGO showed that different pathways were found to be significantly ($p < 0.05$) modulated in both the GSTT1*0 wildtype and deletion variants, with most of them involved in the cellular processes immune response, development, and apoptosis.

[#]Number of significantly modulated genes per defined subgroup:

1) GSTT1*0 wildtype: subjects carrying the GSTT1*0 wildtype variant
2) GSTT1*0 deletion : subjects carrying the GSTT1*0 deletion variant

n= number of subjects per group

Table 4. Metacore™ Analysis of XRCC1*4 Wildtype and Heterozygous and Homozygous Variants Following T-Test Assay : GeneGo Ranking of Biological Pathways Significantly Modulated in Lymphocytes Of Study Subjects Consuming 1 Litre of Blueberry-Apple Juice Daily for 4 Weeks

Process	Pathways Involved	Number of significant genes per group#		Total No of Genes in Pathway
		XRCC1*4 wildtype (n=57)	XRCC1*4 heterozygous and homozygous (n=91)	
Development	Beta-adrenergic receptors transactivation of EGFR	4		**23**
	Regulation of epithelial-to-mesenchymal transition (EMT)	7		**54**
	Role of IL-8 in angiogenesis		18	**35**
	Activation of astroglial cells proliferation by ACM3		10	**16**
	EGFR signaling via PIP3		9	**15**
	Endothelin-1/EDNRA transactivation of EGFR		12	**23**
	A2A receptor signaling		12	**23**
Immune Response	Classical complement pathway	5		**27**
	Lectin induced complement pathway	5		**30**
	Bacterial infections in normal airways	5		**32**
	T cell receptor signaling pathway		16	**34**
	IL-9 signaling pathway		12	**24**
Apoptosis and survival	NO synthesis and signaling	5		**27**
	Granzyme A signaling		13	**22**
Cholesterol and Sphingolipids transport	Recycling to plasma membrane in lung (normal and CF)	3		**9**
	Generic schema (normal and CF)	1		**2**
	Cholesterol metabolism	2		**10**

	Sphingolipid metabolism		17	**33**
	Sphingolipid metabolism / Human version		17	**34**
	Cholesterol Biosynthesis		10	**19**
Cytoskeleton remodeling	Alpha-1A adrenergic receptor-dependent inhibition of PI3K		5	**6**
	Role of PKA in cytoskeleton reorganisation		13	**19**
testosterone biosynthesis and metabolism	Androstenedione and testosterone biosynthesis and metabolism p.3	2		**5**
	Androstenedione and testosterone biosynthesis and metabolism p.3/ Rodent version	2		**5**
	Androstenedione and testosterone biosynthesis and metabolism p.2	2		**6**
	Androstenedione and testosterone biosynthesis and metabolism p.2/ Rodent version	2		**6**

Paired t-test analysis, comparing gene expression profiles before and after the blueberry intervention for each subject, was performed in study population in order to investigate the gene expression effect in specific genetic subgroups which benefit more from the intervention as compared to their genetic counterparts. For XRCC1*4, subjects carrying the wildtype variant showed an overall significant decrease in oxidative DNA damage in ex vivo exposed lymphocytes after the 4-week blueberry intervention.

Analysis by MetacoreTM GeneGO showed that a few different pathways were found to be significantly ($p < 0.05$) modulated in both the XRCC1*4 wildtype as well as heterozygous and homozygous variants, with most of them involved in the cellular processes immune response, development, apoptosis. Modulations in other pathways including Cholesterol and Sphingolipids transport and and testosterone biosynthesis and metabolism were seen for the the XRCC1*4 wt variant while cytoskeleton remodelling was observed in the XRCC1*4 heterozygous and homozygous variants .
Furthermore, the numbers of significantly modulated genes in the pathways were found to be low.

#Number of significantly modulated genes per defined subgroup:

1) and XRCC1*4 wildtypes: subjects carrying the XRCC1*4 wildtype variant
2) and XRCC1*4 heterozygous and homozygous: subjects carrying the XRCC1*4 heterozygous and homozygous variants

n= number of subjects per group

Table 5. Metacore™ Analysis of GSTT1*0 Wildtype and Deletion Variants Following Correlation Assay: Genego Cellular Processes and Biological Pathways Significantly Modulated in Lymphocytes of Study Subjects Consuming 1 Litre Of Blueberry-Apple Juice Daily for 4 Weeks

Process	Pathways Involved	Number of Significant Genes per Group#		Total number of Genes in Pathway
		GSTT1*0 wildtype (n=122)	GSTT1*0 deletion (n=25)	
Immune Response	Role of DAP12 receptors in NK cells	10		37
	HMGB1 release from the cell	6		24
	CD16 signaling in NK cells	8		39
	PGE2 in immune and neuroendocrine system interactions	4		12
	IFN gamma signaling pathway	7		36
	HMGB1/TLR signaling pathway	6		30
G-protein signaling	Rap2A regulation pathway		3	6
	Rap2B regulation pathway		2	3
	RhoA regulation pathway		6	27
	G-Protein alpha-12 signaling pathway		5	21

The outcome of the pathway analysis using MetaCore™ following the "Correlation Assay" is presented here . Spearman's rank correlation analysis was performed to investigate whether the transcriptomic response could be linked to the difference in oxidative DNA damage as measured by the comet assay. Genes in these pathways have also shown a linear response in relation to the difference in oxidative DNA damage.

#Number of significantly modulated genes per defined subgroup:

1) GSTT1*0 wildtypes: subjects carrying the GSTT1*0 wildtype variant
2) GSTT1*0 deletion : subjects carrying the GSTT1*0 deletion variant

n= number of subjects per group

Table 6. Metacore™ Analysis of XRCC1*4 Wildtype and Heterozygous and Homozygous Variants Following Correlation Assay: Genego Cellular Processes and Biological Pathways Significantly Modulated in Lymphocytes of Study Subjects Consuming 1 Litre of Blueberry-Apple Juice Daily For 4 Weeks

Process	Pathways Involved	Number of Significant Genes per Group#		Total Number of Genes in Pathway
		XRCC1*4 wildtype (n=57)	XRCC1*4 heterozygous and homozygous (n=91)	
Immune response	Inhibitory action of lipoxins on superoxide production induced by IL-8 and Leukotriene B4 in neutrophils	7		21
	TLR signaling pathways	9		33
	CD137 signaling in immune cell	7		24
	IL7 signaling in T lymphocytes	7		24
Metabolism	Glutathione metabolism	9	7	31
	Glutathione metabolism / Human version	9	7	32
	Glutathione metabolism / Rodent version	9	7	32
Apoptosis and survival	Inhibition of ROS-induced apoptosis by 17beta-estradiol	7		23
	Anti-apoptotic action of Gastrin	6	4	24
	NO synthesis and signaling	7		27

The outcome of the pathway analysis using MetaCore™ following the "Correlation Assay" is presented here . Spearman's rank correlation analysis was performed to investigate whether the transcriptomic response could be linked to the difference in oxidative DNA damage as measured by the comet assay. Genes in these pathways have also shown a linear response in relation to the difference in oxidative DNA damage. #Number of significantly modulated genes per defined subgroup:

1) XRCC1*4 wildtypes: subjects carrying the XRCC1*4 wildtype variant;
2) XRCC1*4 heterozygous and homozygous : subjects carrying the XRCC1*4 heterozygous and homozygous variants n= number of subjects per group

Table 7- List of Significantly Down- and Up- Regulated Genes (p-value < 0.05) Significantly Modulated Pathways Identified by GeneGo Ontology Map rankings:

Polymorphism	Down-Regulated Genes	Up-Regulated Genes	Biological Pathway	Cellular Process
GSTT1*0 wildtypes	_____	TNF alpha, 1L, 1 beta, TLR4,NF-*k*B,COX1,G protein beta gamma	Immune response	PGE2 in immune and neuroendocrine system interactions
	_____	IL-1 beta,TNF-alpha,TLR-4,NF-*k*B,calmudlin,PKC-beta,P13k cat	Immune response	HMGB1 release from the cell
	TRAF6,Ubiquitin	TLR4, MEK3(MAP2K3),IL1 beta,TNF-alpha, IL1RN	Immune Response	HGMB1/TLR signaling pathway
	NKG2A,CD94,CD3zeta, IgG1,PLC- gamma 2,NF AT1(NFATC2),FASL(TNFSF6)	p13k cat class 1A, PLA2, TNF alpha,Calmudulin	Immune Response	CD16 signaling in NK cells
	PLC gamma 2, c-MYC	p13kcat class 1A,soc s1,calmodulin,icam1,p21,CBP	Immune Response	IFN gamma signaling pathway
GSTT1*0 deletion	G-protein alpha s, M-Ras	RAP-2A	G-protein signaling	Rap2A regulation pathway
	M-Ras	RAP-2B	G-protein signaling	Rap2B regulation pathway
	FAK1,LARG,Gprotein alpha q 11,RhoG01 alpha	D1A1,PLD1,IGF-1 receptor	G-protein signaling	RhoA regulation pathway
	LARG, RASA2, R-Ras, M-Ras, MEKK1(MAP 31k)	G-protein beta gamma	G-protein signaling	G-Protein alpha-12 signaling pathway
	G-protein alpha q, PLC-beta, G-protein alpha s	G-protein bea gamma 2, G-protien alpha 11, adenylate cyclase, PKC-beta	Cytoskeleton remodelling	Thyroliberin in cytoskeleton remodelling

	G-protein alpha q, G-protein alpha s, PLC-beta, MEF 2A, MEF 2, NCX1	G-protein beta gamma, G-protein alpha, PKC beta	Cardiac Hyperthrophy	Ca2+ dependent NF-AT signaling
XRCC1*4 wildtypes	_____	CD14, TLR4,TLR6,MD-2,CD14,TLR8,MYD88,TOLLIP,IRAKM,NF-*k*B,MEK3(MAP2K3),TLR8	Immune response	TLR signaling pathways
	ATF-2Cjun,ATF-2,BCL-2	BIM,BFLI,CYCLin D2, NF-*k*B,MEK6,MEK3	Immune response	CD137 signaling in immune cell
	LCK,BCL-2,FKHR,GLUT1	JAK3,RS-2,CBP	Immune response	IL7 signaling in T lymphocytes
	GCL cat,GPX2 homotetramer	OPLA homodimer,GGT1, MGST2 homotrimer, MGST3,GPX1 homotetramer	Metabolism	Glutathione metabolism
	GCL cat,GPX2 homotetramer	AMPN, OPLA homodimer,GGT1, MGST2 homotrimer, MGST3,GPX1 homotetramer	Metabolism	Glutathione metabolism / Human version
	GCL cat,GPX2 homotetramer	AMPN, OPLA homodimer,GGT1, MGST2 homotrimer, MGST3,GPX1 homotetramer	Metabolism	Glutathione metabolism / Rodent version
XRCC1*4 Heterozygous and Homozygous	GCL cat	GGT1,GSTM2 homodimer,GSTM3,GSTA4,GPX7,GSTA2 homodimer	Metabolism	Glutathione metabolism
	GCL cat	GGT1,GSTM2 homodimer,GSTM3,GSTA4,GPX7,GSTA2 homodimer	Metabolism	Glutathione metabolism / Human version
	GCL cat	GGT1,GSTM2 homodimer,GSTM3,GSTA4,GPX7,GSTA2 homodimer	Metabolism	Glutathione metabolism / Rodent version

This table lists the modulated Genes (p- value < 0.05) in Significant Pathways as were Identified in GSTT1*0 wildtype and Deletion and XRCC1*4 wildtype using Metacore^{TM} GeneGo Ontology Map rankings. Down- and Up-regulated genes in each significantly modulated biological pathways and cellualr process are indicated.

Table 8- Network Lists of GSTT1* 0 Wildtypes and Deletions and XRCC1*4 Wildtypes identified by Metacore™ GeneGo analysis using Shortest Paths Networks

Gene Variant	Network	GO processes	p-Value
GSTT1*0 wildtype	p21, CBP, TRAF6, RelB (NF-*k*B subunit), NOL3	regulation of cell death (72.5%; 3.508e-24), regulation of apoptotic process (70.0%; 3.630e-23), regulation of programmed cell death (70.0%; 4.513e-23), induction of apoptosis (50.0%; 2.006e-22), induction of programmed cell death (50.0%; 2.335e-22)	**1.540E-13**
GSTT1*0 wildtype	Calmodulin, Caspase-3, FasR(CD95), PLC-gamma 1, FADD	lipid catabolic process (40.8%; 7.416e-26), phospholipid metabolic process (40.8%; 6.174e-25), organophosphate metabolic process (40.8%; 2.550e-24), positive regulation of molecular function (63.3%; 3.246e-23), platelet activation (36.7%; 2.697e-20)	**2.450E-08**
GSTT1*0 deletion	MEKK1(MAP3K1), FAK1, NCX1, MEF2A, PKC-beta	intracellular signal transduction (61.4%; 4.268e-19), wound healing (47.7%; 5.140e-19), platelet activation (36.4%; 4.274e-18), regulation of body fluid levels (45.5%; 7.842e-18), response to external stimulus (56.8%; 1.446e-17)	**1.440E-28**
GSTT1*0 deletion	M-Ras, PLD1, LARG, IGF-1 receptor, RASA2	response to peptide hormone stimulus (56.0%; 1.366e-30), response to endogenous stimulus (70.0%; 1.411e-29), response to hormone stimulus (64.0%; 4.296e-28), response to organic substance (78.0%; 9.738e-27), nerve growth factor receptor signaling pathway (40.0%; 1.903e-25)	**2.260E-22**
XRCC1*4 wildtype	Bcl-2, TLR4, CBP, RelB (NF-*k*B subunit), Bim	positive regulation of metabolic process (77.1%; 1.261e-20), positive regulation of macromolecule metabolic process (74.3%; 2.361e-20), positive regulation of biological process (88.6%; 4.512e-19), positive regulation of cellular metabolic process (68.6%; 4.370e-17), innate immune response (45.7%; 2.392e-16)	**3.530E-40**
XRCC1*4 wildtype	ATF-2, MyD88, Lck, MEK6(MAP2K6), PLD1	response to stress (80.9%; 9.812e-23), response to wounding (57.4%; 3.073e-21), immune response-activating signal transduction (34.0%; 2.250e-19), immune response-regulating signaling pathway (34.0%; 5.298e-19), regulation of protein metabolic process (57.4%; 3.477e-18)	**7.810E-32**

Gene networks for the significantly modulated biological processes, as identified by Metacore™ GeneGo analysis using shortest path networks, are generated for GSTT1*0 wildtypes and deletion and for XRCC1*4 wildtypes in MetaCore™ in order to visualize the complex set of connections between the genes and their intercations.

The values in brackets indicate the percent of involvement in the Metacore™ GeneGo processes and the related p-value .

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Interindividual Differences in Response to Blueberry Juice Intervention in Healthy Human Subjects: A Genomics Approach

Richting: **master in de biomedische wetenschappen-milieu en gezondheid**
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Hosseinzadeh, Sharareh

Datum: **19/06/2012**