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Milan, March 4, 2022

Antonio Pizzarella

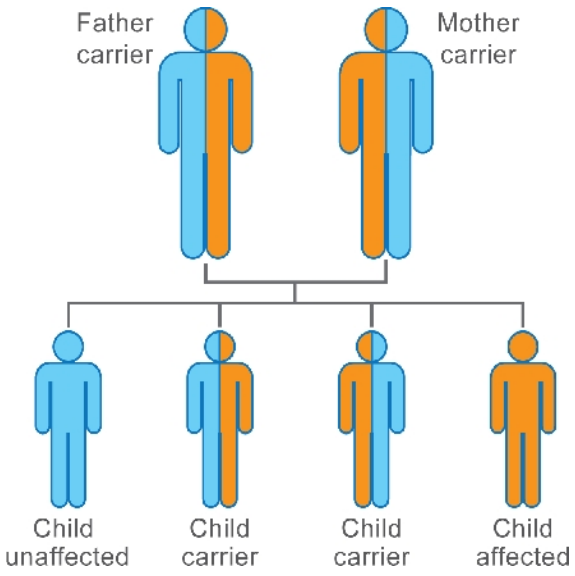
Toxic Element Clearance Profile

There is NO significant intoxication, except relative to Gadolin, which is often used in imaging, but may not unfavorably influence the current clinical picture. Useful reinforce zinc and glutathione intake in this regard, but ideally for the latter would be to take advantage of the intravenous route.

DNA testing - commentary

LEGEND

As explained earlier, the indication of increased risk (unfavorable variants) is based on statistical studies that associate the incidence of the disorder or dysmetabolism with the individual genotype. Increased risk, however, does not necessarily imply the occurrence of a disease directly associated with it.



Reference/"normal" type: NO ALLELE (CARRIER) RECAIMS THE SNP

Reference/"heterozygous" type: ONE ALLELE (CARRIER) RECAIMS THE SNP

Reference / "homozygous" type: BOTH ALLELES (CARRIERS) BEAR THE SNP

A **responsive variant** is defined as a genotype associated with ascertained gene/environment interactions and allows for the identification of the specific action (lifestyle, physical activity, diet, etc.), which is most appropriate in relation to the genetic set-up.

Favorable variant means a genotype associated with positive/protective health effects.

Unfavorable variant (*risk/risk*) refers to a genotype directly associated with health risks and/or imbalances in the body's physiological functions and implies the need to identify early preventive measures and/or the clinical need for further diagnostic investigation.

Example:

GENE	GENOTYPE	RESPONSIVE	PROTECTIVE	RISK
SNP-X Example: G>A Pos. -75 Promoter	**(G/G)** NO ALLELE CARRIES SNP	okay	okay	!

Finally, it is important to remind you how each individual gene may present unfavorable, favorable, or no clinical consequence polymorphisms. **The important thing then is the overall evaluation of the whole picture of the polymorphisms analyzed, assigning to each of them the "right" statistical weight, in order to arrive, by the specialist, at a final interpretation of the genomic test performed.**

Some symbols placed at the susceptibilities under consideration summarize the status:

FAVORABLE CONDITION: NO RESTRICTIONS

PARTIALLY UNFAVORABLE CONDITION: PAY ATTENTION

UNFAVORABLE CONDITION: ACTIVELY INTERVENE

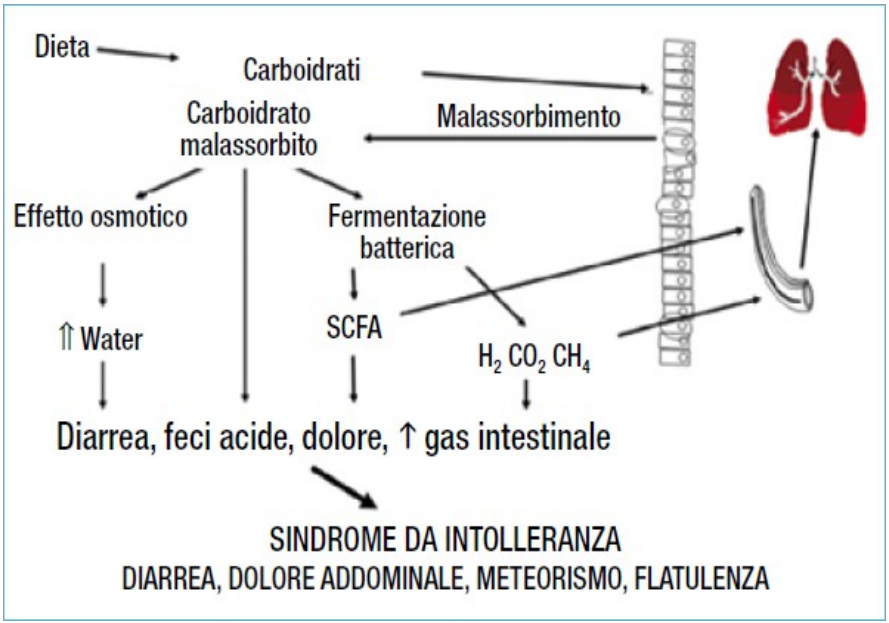
SCIENTIFIC REPORT

The most important aspects that emerged with the genomic analysis you performed are highlighted for you below; a more analytical explanation will follow.

NUTRITIONAL INTOLERANCES AND SENSITIVITIES

- **Absence of increased genetic susceptibility toward gluten intolerance.**
- **Slow and therefore NOT physiological metabolization of caffeine, with the presence of hypersensitivity to it. Slow metabolizer.**
- **Slow and therefore NOT physiological metabolization of alcohol, resulting in hypersensitivity to it. Slow metabolizer.**
- **Moderate increase in sensitivity toward salt.**
- **PRESENCE of primary lactose intolerance, from deficiency in lactase biosynthesis. This intolerance is frequently involved in colonic problems because of the intestinal malabsorption it induces and the extra work required of the intestinal microbial flora (see figure).**

- Lactose intolerance exposes one to an increased risk of calcium , which negatively affects bone metabolism.
- Lactose intolerance, in case of concomitant frequent intake of its contributing foods, induces easier weight gain.
- Lactose intolerance induces conditions of widespread bloating (abdominal meteorism) and is often conducive to water retention.
- Lactose intolerance is conducive to gastro-enteropathies and reinforces cephalgic conditions.
- Lactose intolerance contributes to a feeling of widespread malaise also accompanied by a sense of fatigue.



DETOXIFICATION - OXIDATION - INFLAMMATION

- Overall physiological detoxifying and detoxifying capacity.
- There is only suboptimal function of phase I hepatic detoxification. This results in easier activation of inhalation, topical rather than oral contact environmental carcinogens.
- Efficiency of function related to phase II liver detoxification, with physiological elimination of carcinogens introduced or formed in the body, due to

Of the absence of deletion within GSTM1 and GSTT1, important detox enzymes in the body.

- Physiological antioxidant capacity within SOD2.
- Overall, there is a marked tendency toward inflammaging, linked to genetic tendency toward reduced biosynthesis of anti-inflammatory molecules (interleukin-10), which can be likened to "firefighters" ready to extinguish fires ignited in the body.
- There is thus a genetic tendency toward a marked pro-inflammatory imbalance, resulting in increased susceptibility toward related diseases: ischemic heart disease, gastro-enteric diseases, osteo-articular diseases, neuro-inflammation and associated cognitive decline, and nonspecific neoplasms (see figure). Essential to keep omega6/omega3 ratio monitored, through eicosanoid testing or omega screening. Also indicated is an "anti-inflammatory" diet.

NUTRIENT SENSITIVITY

- Moderate increase in genetic predisposition toward dyslipidemic conditions.
- NOT physiological function of the vitamin D receptor.
- Absence of significant increase in carbohydrate sensitivity nor of increased genetic predisposition toward forms of insulin resistance nor toward type 2 diabetes.

- **Presence of genetic tendency toward forms of hyperhomocystinemia, which in case of values above 13 umol/l would lead to significantly increased risk of stroke and cardiovascular disease; folate and vitamin B metabolism NOT physiological.**

CLINICAL COMMENTARY

FOOD INTOLERANCES AND SENSITIVITIES

LATTASI **T>C POS. -13910**

GENERAL INFORMATION

Lactose intolerance is the inability to digest significant amounts of lactose, the sugar found in the greatest quantity in milk. **Lactose intolerance is caused by shortages of the enzyme lactase produced by cells exposed on the surface of small intestine**. Lactase breaks down lactose into two simpler sugar forms called glucose and galactose, which are then absorbed into the bloodstream. Without lactase, the lactose in milk cannot be broken down and thus absorbed. Lactose cannot easily pass through the wall of the intestine into the bloodstream, so it remains in the intestine. Quickly, intestinal bacteria begin to metabolize lactose. **In doing so, they produce large amounts of gas through fermentation. The gas causes a number of unpleasant abdominal symptoms such as stomach cramps, bloating, flatulence, and sometimes diarrhea**.
Genetic testing for lactase deficiency can complement indirect methods for individual determination of risk for lactose malabsorption and osteoporosis. Genetically determined lactose intolerance in adulthood affects more than 30 percent of the Italian population. In addition**polymorphism analysis makes it possible to distinguish between lactose intolerance typical of adulthood and the form induced secondarily by a given environment, such as lactose intolerance as a result of particular gastrointestinal diseases and/or exposure to intestinal parasites**.

GENOTYPIC ASSOCIATION FOUND

GENE	GENOTYPE	RESPONSIVE	PROTECTIVE	RISK
LCT T>C Pos. -13910	**MUT : (C/C)** BOTH ALLELES CARRY SNP			**!!**

COMMENT

A C/C genotype was found in your case: therefore, there is a genetic lactose intolerance. Important therefore to eliminate the presence of milk as well as its derivatives (yogurts and dairy products mainly).

In general, therefore, try to avoid the use of dairy products that contain lactose (milk, butter, some fresh and lightly aged cheeses, etc.), as well as the use of non-dairy products that may contain lactose as a food additive (whey, dried milk residues, modified dairy ingredients, etc.).

When one cannot avoid lactose intake or consciously chooses to take lactose, the use of lactase enzyme supplements, as well as treatment with lactic acid ferments, is recommended. This condition, if neglected, easily evolves IN TIME into intestinal dysbiosis, resulting in problems with dyspepsia, heavy stomach feeling and postprandial drowsiness, as well as meteorism (abdominal bloating) and irregularity of bowel movements. Frequent consumption of lactose-containing products would be more likely to result in weight gain.

In relation to the findings, for the purpose of effective benefit, elimination of lactose-containing foods would still be indicated. Remember, however, that traditional cheese as well as grana padano, where aged over 30 months, are almost lactose-free.

It should be remembered, however, that all types of milk on the market (cow, goat, sheep, buffalo, skim or whole, powder or paste) and all dairy products (fresh and lightly ripened cheeses, yogurt, ice cream, cream, fiordilatte) contain lactose, except for particular qualities. In addition to these, there are foods that contain lactose even if you would not expect it, such as margarine, white bread, salad dressings, some candies, cured meats (especially bologna and bacon), cookies, and other sweets. If present in trace amounts, it is NOT a problem.

NOTE:

PREVALENCE OF DNA TEST VERSUS BREATH TEST IN THE DIAGNOSIS OF PRIMARY LACTOSE INTOLERANCE BY LACTASE DEFICIENCY
BRIEF INTERNATIONAL LITERATURE.

1. ***Br J Nutr. 2010 Sep;104(6):900-7. Excellent agreement between genetic and hydrogen breath tests for lactase deficiency and the role of extended symptom assessment. Pohl D, Savarino E, Hersberger M, Behlis Z, Stutz B, Goetze O, Eckardstein AV, Fried M, Tutuian R. Abstract Conclusion. Genetic testing has an excellent agreement with the standard lactose H2-BT, and it may replace breath testing for the diagnosis of LD.***
2. ***Rev Med Chil. 2012 Sep;140(9):1101-8. Comparative performance of symptoms questionnaire, hydrogen test and genetic test for lactose intolerance. Rollán A, Vial C, Quesada S, Espinoza K, Hatton M, Puga A, Repetto G. Abstratc Conclusion: genotype C/C_13910 is responsible for hypolactasia in this population. H2 and genetic tests are simple and similarly accurate to diagnose lactose intolerance in adults.***

3. *Acta Cir Bras. 2013;28 Suppl 1:77-82. Comparison of Quick Lactose Intolerance Test in duodenal biopsies of dyspeptic patients with single nucleotide polymorphism LCT-13910C>T associated with primary hypolactasia/lactase-persistence. Mattar R, Basile-Filho A, Kemp R, dos Santos JS. Abstract Conclusion: quick test is highly sensitive and specific for hypolactasia diagnosis and indicated those patients with symptoms of lactose intolerance.*

ALCOHOL SENSITIVITY — ADH1c

GENERAL INFORMATION

Sensitivity to alcohol depends on the rate at which alcohol is metabolized by the enzyme alcohol dehydrogenase, which converts it into the toxic compound acetaldehyde, which is in turn transformed into the nontoxic compound acetate by another enzyme called aldehyde dehydrogenase: Alcohol Ethyl-->Acetaldehyde-->Acetate.

Acetaldehyde, in addition to causing DNA damage and being carcinogenic, is responsible for the ill effects of excessive alcohol consumption. People with an ADH1C gene variant are slow metabolizers and tend to have higher blood alcohol levels following alcohol ingestion.

GENOTYPIC ASSOCIATION FOUND

GENE	GENOTYPE	RESPONSIVE	PROTECTIVE	RISK
ADH1C Ile349Val	WT : (Ile/Ile) NO ALLELE CARRIES SNP		okay	

COMMENT

Genetic testing showed the physiological genotype, which quickly metabolizes alcohol, consequently there is NO hypersensitivity to it.

CAFFEINE SENSITIVITY

GENERAL INFORMATION

One of the substances most metabolized by CYP1A2 **is caffeine** (1,3,7-trimethylxanthine). Caffeine, which is the most widely consumed stimulant worldwide, is metabolized predominantly by cytochrome P450 1A2 (CYP1A2) in the liver. CYP1A2 is responsible for approximately 95% of caffeine metabolism and demonstrates great individual variability in enzyme activity. A polymorphic substitution

in pos. -163 of the CYP1A2 gene **decreases the inducibility of the enzyme, as measured by the ratio of caffeine plasma or urine to caffeine metabolites after taking a dose of caffeine, and causes decreased caffeine metabolism**.

Coffee is a primary source of caffeine and is involved in the development of cardiovascular diseases such as acute myocardial infarction (AMI). Cornelis et al. conducted an extensive study (2014 cases with a first nonfatal IMA and 2014 controls) to determine whether the C>A pos. -163 polymorphism changes the association between coffee consumption and the risk of acute, nonfatal IMA. Well, they found that **coffee intake was associated with increased risk for acute IMA only in individuals with low caffeine metabolism**, a fact that suggests that caffeine plays an important role in this association.

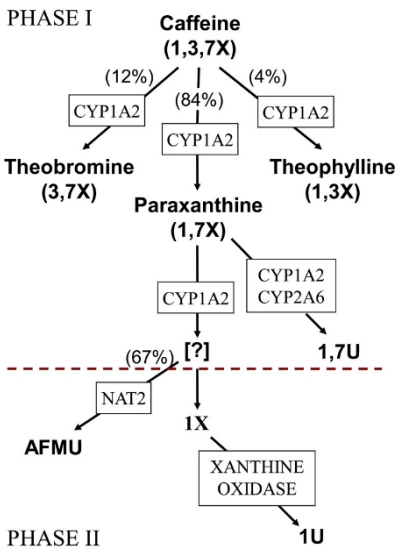
GENOTYPIC ASSOCIATION FOUND

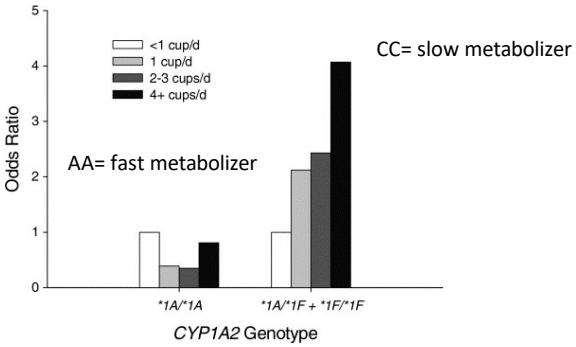
GENE	GENOTYPE	RESPONSIVE	PROTECTIVE	RISK
CYP1A2 C>A Pos. -163	**WT : (A/A)** NO ALLELE CARRIES SNP		**okay**	

COMMENT

The homozygous physiological A/A genotype was found to allow rapid metabolization of caffeine; therefore, there is no hypersensitivity to it.

CYP1A2 and Caffeine Metabolism





Individuals who are **homozygous or heterozygous for the CYP1A2*1F** allele are 'slow' caffeine metabolizers.

Thus for these individuals increased intake of caffeine seems to be associated with a concomitant increase in the risk of **non7fatal myocardial infraction (MI).**

GB GIDARO 4/7/2015

Brief bibliographical citation

Palatini P et al. ***CYP1A2 genotype modifies the association between coffee intake and the risk of hypertension.*** *J Hypertens. 2009 Aug;27(8):1594-601.*

Cornelis MC et al. ***Coffee, CYP1A2 genotype, and risk of myocardial infarction.*** *JAMA. 2006 Mar 8;295(10):1135-41.*

SALT SENSITIVITY -

GENERAL INFORMATION

Angiotensin-converting enzyme (ACE) degrades bradykinin, a cardiac protective peptide, and plays an important role in regulating blood pressure and balancing electrolytes hydrolyzing angiotensin I to angiotensin II. Angiotensin II is a potent vasopressor and a peptide that stimulates aldosterone, maintaining cardiovascular homeostasis. The human ACE gene carries an insertion (I) / deletion (D) polymorphism. **The I/I genotype is associated with high plasma levels of ACE, which increases angiotensin II concentration. This fact is reflected in a greater genetic predisposition for hypertension. Genotype I/I is further considered as a (weak) genetic risk marker for myocardial infarction and left ventricular hypertrophy.**

GENOTYPIC ASSOCIATION FOUND

GENE	GENOTYPO	RESPONSIVE	PROTECTIVE	RISK
ACE Ins>Del Intron 16	**MUT : (Del/Del)** BOTH ALLELES CARRY THE FAVORABLE SNP		okay	

COMMENT

The unfavorable allelic variant I was NOT found in his DNA; therefore, there is NO hypersensitivity to salt. This condition favors nutritional control of blood pressure. Therefore, it is a mutation in this favorable context.

Brief bibliographical citation

Giner V et al. ***Renin-angiotensin system genetic polymorphisms and salt sensitivity in essential hypertension.*** *Hypertension. 2000 Jan;35(1 Pt 2):512-7.*

GLUTEN

GENERAL INFORMATION

Celiac disease is a lifelong intolerance to gliadin, the soluble alcohol fraction of gluten, which is a collection of proteins found in grains such as wheat, barley, oats, rye, millet, kamut, and spelt.

Gluten intolerance causes in the small intestine a progressive atrophy of the intestinal villi, structures deputed to nutrient absorption, leading to a picture of malabsorption, the severity of which is proportional to the extent of damaged intestinal surface area and the intensity intolerance. The

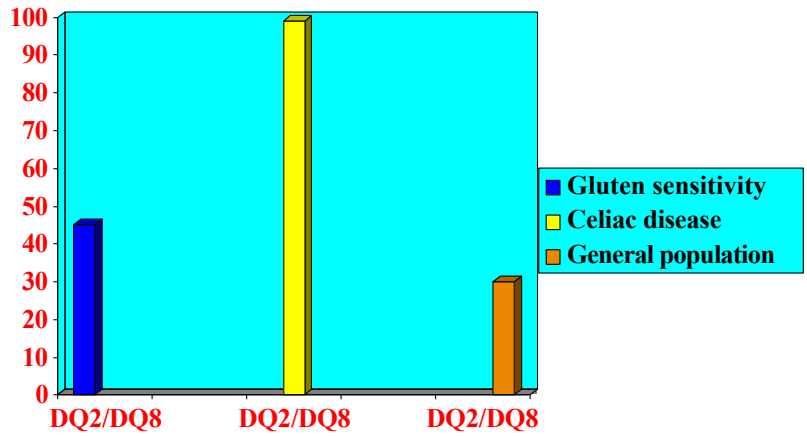
causes are both genetic and environmental.

From a genetic point of view, celiac disease is associated with the presence of the HLA-DQ2 and HLA-DQ8 genes. Ninety percent of patients with celiac disease have the HLA-DQ2 gene; however, a minority of patients have the HLA-DQ8 gene.

Positivity to DQ2 or DQ8 does not mean disease diagnosis, but an increased likelihood of having the actual celiac disease.

Negativity to DQ2 or DQ8, on the other hand, means such a low probability of developing the disease that it pemits the diagnosis of celiac disease or gluten intolerance to be ruled out.

Positivity to only one of the two genes, frames a sort of intermediate form, in which there would still be some sort of hypersensitivity to gluten, which should consequently be taken by reducing its frequency and load.



See the following proceedings for more details:

June 21, 2011 Session: Gluten sensitivity Chairs Katri Kaukinen, FI - Bana Jabri, US
Joe Murray, US Introduction
Detlef Schuppan, US Molecular basis of wheat induced innate immune responses
Jessica Biesikierski, AU Self-diagnosis of non-coeliac gluten intolerance by Australian adults
Margit Brottveit, NO Influence of personality on gluten induced symptoms in coeliac disease and non-coeliac gluten intolerance.
Umberto Volta, IT Serological tests in gluten sensitivity (non celiac gluten intolerance)
Anna Sapone, EN Antibodies against alpha gliadin (AGA) and deamidated gliadin peptides (DGP) in patients with autistic spectrum disorder (ASD)

GENOTYPIC ASSOCIATION FOUND

POSITIVE APLOTIPE: presence of the DQ2 heterodimer.

COMMENT

Genetic testing indicates that a predisposition to celiac disease rather than a condition of simple "gluten sensitivity" cannot be ruled out. In fact, with this genotype, 1 in 35 people develop celiac disease, while in Italy the estimated average is 1 in 100/150 people.

Basically, therefore, there is not a true celiac disease condition, but still a moderate increased risk of incurring a kind of reduced tolerance, compared to the average population, toward gluten. Today this reality is framed

under the term "gluten sensitivity," which defines precisely those conditions of serologic negativity and genomic positivity.

Specifically, gluten sensitivity, also referred to as non-celiac gluten sensitivity, is a syndrome characterized by multiple gastro-intestinal symptoms (abdominal meteorism, postprandial heaviness, slow digestion, dyspepsia, heartburn, etc.) and/or extra-intestinal symptoms (anemia, osteopenia, muscle cramps, diffuse arthralgias, dermatitis, hair loss, headaches, etc.) occurring within a short time after the intake of foods containing significant amounts of gluten and improving or disappearing after elimination or reduction of gluten in subjects in whom the diagnosis of celiac disease has been ruled out on the basis of serology negativity and/or demonstration of normal intestinal mucosa, as well as the diagnosis of wheat allergy by negativity of IgE and wheat-specific Prick tests.

It follows that there is a need not for its total elimination, but simply to moderately reduce its intake (load) and frequency. Indeed, the symptomatology is insidious and sometimes manifests its negative consequences later in life, even simply as late peripheral neuropathy.

INFLAMMAGING - DETOXIFICATION - ANTIOXIDANT CAPACITY

TUMOR NECROSIS FACTOR alpha

GENERAL INFORMATION

The tumor necrosis factor-alpha (TNFα) gene, **encodes for a pro-inflammatory cytokine that is pleiotropic i.e., capable of performing numerous regulatory functions on immune responses**. TNFα is also an important mediator of both acute and chronic inflammatory responses. The concentration of TNFα increases during vascular damage produced by thrombus formation; this factor **promotes damaged endothelial cells by stimulating them to produce adhesion molecules. Thus by promoting adhesion to endothelial cells TNFα behaves as a factor promoting atherogenesis** and vascular damage causing infarction. TNF is also involved in a great many processes, such as apoptotic cell death, proliferation, differentiation, carcinogenesis, and viral replication.

GENOTYPIC ASSOCIATION FOUND

GENE	GENOTYPO	RESPONSIVE	PROTECTIVE	RISK
TNF	WT : (G/G) NO ALLELE CARRIES SNP		okay	

COMMENT

Tumor necrosis factor (usually abbreviated as TNF, from Tumor necrosis factor) is an interleukin involved in systemic inflammaging and is a member of a group of cytokines that stimulate the so-called acute phase reaction. TNF is involved in a great many processes such as cell death, proliferation, differentiation, carcinogenesis, and viral replication.
This unfavorable polymorphism was NOT found in His DNA. Therefore, the immunological profile is not adversely affected.

INTERLEUKIN 10 G>A POS. -1082

GENERAL INFORMATION

cytokine interleukin-10 (IL-10) physiologically **limits** inflammation and **lowers its levels**. Age-related diseases begin and worsen as a result of systemic inflammation; in contrast, genetic variations that result in increased production of anti-inflammatory cytokines have been seen in association with serene aging: a polymorphism in the promoter region has been seen to regulate IL-10 levels. An adenine (A) at -1082 in the promoter region of the gene for IL-10 is associated with low IL-10 production, while a guanine (G) is with high IL-10 production.

GENOTYPIC ASSOCIATION FOUND

GENE	GENOTYPO	RESPONSIVE	PROTECTIVE	RISK
IL10 G>A Pos. -1082	**MUT : (A/A)** BOTH ALLELES CARRY THE UNFAVORABLE SNP			!!

COMMENT

Interleukin IL-10 regulates inflammatory responses and exhibits immunosuppressive activity. Allelic variant in the form of markedly unfavorable homozygous (A/A) genotype has been detected in His DNA.
In fact, the A/A genotype leads to a NON-physiological ability to synthesize anti-inflammatory interleukins, the so-called "firefighters" that can be called upon to extinguish fires that might be ignited in one's body and that, given this genetic profile, tend to be produced in reduced amounts, compared to the population average, consequently promoting inflammaging.

INTERLEUKIN 6 G>C POS. -174

GENERAL INFORMATION

Interleukin-6 (IL-6) is a multifunctional cytokine and is involved in the amplification sdof the inflammatory response. It has been suggested that a polymorphism in the promoter region (G>C Pos. -174) modulates plasma levels of IL6. **In the case of their increase beyond physiological levels, there would be an excessive inflammatory response toward pathogenic noxae. This condition would result, if not modulated by other enzymatic factors, in a pro-inflammatory background, detrimental to health and individual life expectancy.**

GENOTYPIC ASSOCIATION FOUND

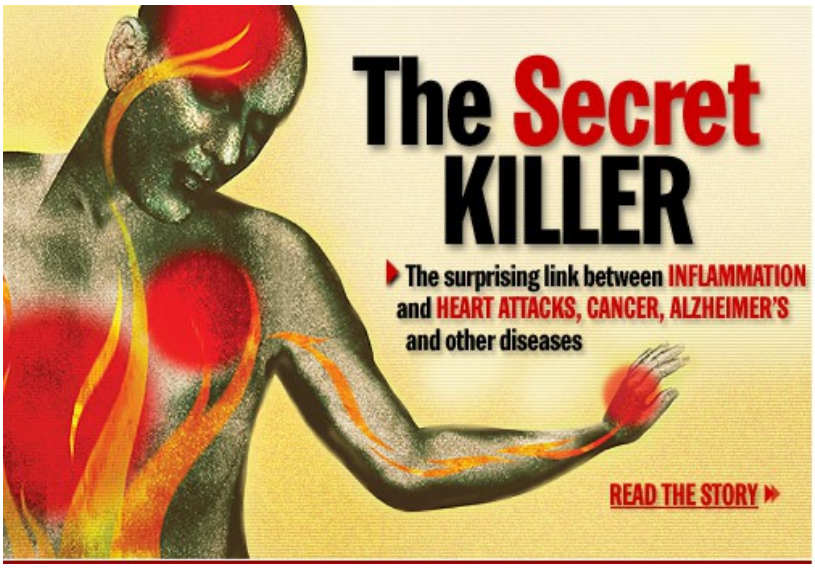
GENE	GENOTYPO	RESPONSIVE	PROTECTIVE	RISK
IL6 G>C Pos. -174	**HET : (G/C)** ONE ALLELE CARRIES THE UNFAVORABLE SNP			!

COMMENT

The mildly unfavorable heterozygous variant was found in His DNA, which results in a tendency toward a moderately more pronounced inflammatory response than the population average, resulting in a per se increased susceptibility toward inflammaging and body attrition.

It is as if one has a genetic constitution that favors the formation and production of "pyromaniacs," the main "actors" in the inflammatory process, in quantities that are NOT entirely physiological and therefore excessive.

GLOBAL COMMENTARY



Overall, there is an inflammatory imbalance: both a tendency toward excessive and NOT entirely physiological biosynthesis of key pro-inflammatory molecules (IL-6) and a marked

Reduction in the ability to contain inflammatory processes, given the deficiency of "firefighters," i.e., key anti-inflammatory interleukin (IL-10).

Pronounced inflammaging results in increased susceptibility toward cardiovascular inflammatory diseases (cardiac ischemia) and neuroinflammation. Inflammaging also underlies both osteo-articular and neoplastic chronic degenerative diseases. Therefore, it is important to contain its effects.

SOD 2

GENERAL INFORMATION

Free radicals cannot be seen or heard, they are sneaky and silent, yet they pose a major threat to our bodies because they can undermine the integrity of our bodies by exerting real cellular aggression, **called oxidative stress.**

Under optimal conditions, the body of a healthy person defends itself against oxidative stress through its own antioxidant system, through enzymatic (superoxide dismutase, catalase, glutathione) and nonenzymatic mechanisms (vitamin A, vitamin E, vitamin C, carotenoids, bioflavonoids, other substances with antioxidant action). However, these mechanisms are only able to neutralize that small amount of free radicals that are formed physiologically in the cell, while they are insufficient to deal with true oxidative stress.

Specifically, oxidative stress can be defined as a particular type of **chemical** stress, **induced-locally and/or systemically-by** the presence, in a living organism**, of an excess of oxidizing chemical species (SCO),** generally-but not exclusively-oxygen-centered (reactive oxygen species, **ROS**)**, secondary to an increased production of** them and/or **a reduced efficiency** of the physiological **antioxidant defense** systems, which are in charge of their control.

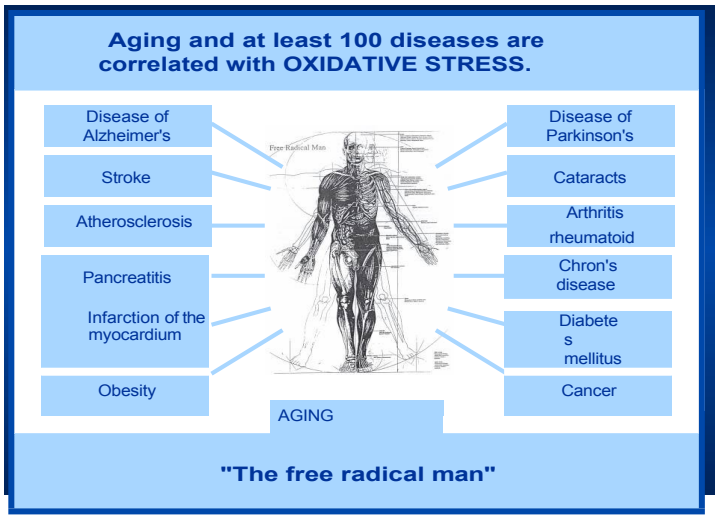
An increase in free radicals beyond the value considered normal therefore produces damage at the cellular level, damage that is not immediate but inevitably manifests itself over time, with impaired cell function. **Various causes** can promote an increase beyond the normal threshold of these free radicals.

GENOTYPIC ASSOCIATION FOUND

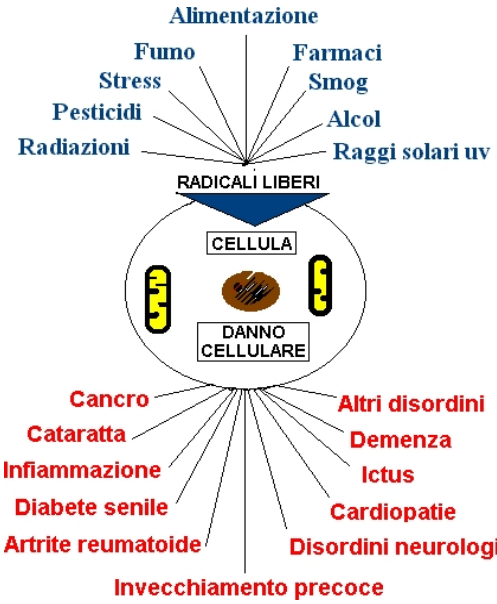
GENE	GENOTYPE	RESPONSIVE	PROTECTIVE	RISK
SOD2	HET : (T/C)			!

COMMENT

His Snip is associated with a slightly weakened antioxidant enzyme function, compared with the population average, due to the unfavorable heterozygous mutation involving the superoxide dismutase (SOD2) gene.



The production of natural "anti-rust" is therefore NOT entirely physiological. From health perspective, one is more susceptible to "rust," i.e., imbalances in the oxidative balance, which contribute to wear and tear on the body (see images).
Antioxidant therapy is indicated, especially during the summer months, and a diet that increases the presence of high-antioxidant foods in the daily diet, as suggested below.



DETOXIFICATION

GENERAL INFORMATION

Glutathione S-transferase M1 (**GSTM1**) is able to detoxify reactive electrophiles that can act as mutagens, and the null genotype of GSTM1 **can increase the effect of carcinogenesis, particularly in case of cigarette smoking habit.**

One of the main phase 2 detoxification reactions is the conjugation with glutathione operated by the enzymes Glutathione-S-Transferase (classes: alpha, mu, pi, theta, and zeta) and leading to the formation of very soluble compounds called mercaptans. This reaction eliminates numerous compounds including carcinogens, drugs, environmental toxins, and products of oxidative stress. **A variation in the GSTM1 and GSTT1 gene, insertion/deletion of most of the gene, (I = insertion, D = deletion), leads to total loss of enzyme function.** Deletion in the **GSTT1 gene** has also been associated with reduced clearance of carcinogens and the process of carcinogenesis.

Susceptibility to toxins and carcinogens in the diet depends largely on the rate at which these substances are metabolized by the enzyme system. **A variation in the CYP1A2*1F gene gives rise to an enzyme with fast activity that increases the bioactivation of carcinogens in food and the risk of cancer**.

GENOTYPIC ASSOCIATION FOUND

GENE	GENOTYPO	RESPONSIVE	PROTECTIVE	RISK
GSTM1 Available> 0 allele	0 allele			!
CYP1A2 -163 A>C	MUT (A/A)			!
GSTT1 Available> 0 allele	I allele		okay	

COMMENT

The body is equipped with an enzymatic detoxification system that neutralizes and eliminates in 3 stages all substances other than nutrients (xenobiotics) such as drugs, toxins, and carcinogens. In stage 1 toxins are chemically modified; in stage 2 they are conjugated with special chemical groups to facilitate their excretion; in stage 3 (excretion) toxic substances are eliminated from the body by urine or bile.

In Phase I detoxification, toxic substances undergo chemical transformations operated by enzymes found primarily in the liver. The main enzyme is cytochrome P450, which exists in multiple forms, including one produced by the CYP1A2 gene (its homozygously altered case, resulting in more rapid activation of environmental and dietary xenobiotics): this enzyme form is involved in the metabolism of toxic and carcinogenic substances, caffeine, and numerous drugs. Some carcinogens such as Polycyclic Aromatic Hydrocarbons (PAHs), to which benzo(a)pyrene (a substance also found in cigarette smoke and automobile exhaust fumes) belongs, are produced in meat and fish as a result of grilling, frying or the smoking process.

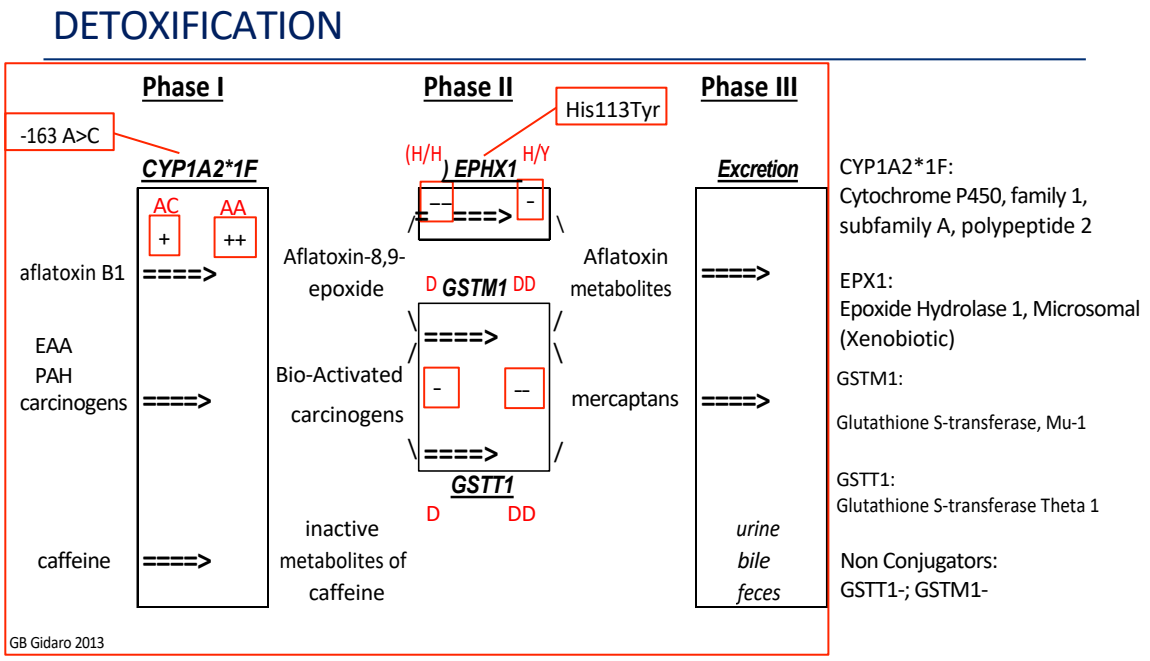
Some reactions activate toxic substances, but the overall effect of all reactions is the elimination of toxic substances. The susceptibility to the accumulation of toxic substances is presented below based on the genetic analysis of the main enzymes involved in the detoxification process and of nutrigenomic interest: CYP1A2*1F and other CYP450 isoforms, GSTM1, GSTT1, GSTP1, EPHX1.

In particular, one of the main detoxification reactions of phase 2 is conjugation with

glutathione operated by glutathione-S-transferase enzymes (classes: alpha, mu, pi, theta, and zeta) and leading to the formation of very soluble compounds called mercaptans. This reaction eliminates numerous compounds including carcinogens, drugs, environmental toxins, and products of oxidative stress.

A variation at the GSTM1, GSTP1, and GSTT1 gene, insertion/deletion of most of the gene, (I = insertion, D = deletion), leads to the total loss of SPECIFIC enzyme function, as found in his case, in which there is deletion of GSTM1.

Because its phase I (alteration in certain isoforms of genes belonging to CYP450) rapidly produces activated molecules and its phase II has a reduced capacity for conjugation and thus elimination, there is easier accumulation of toxic substances.



Shu-Feng Zhou et al. (2009); **Chunyan He** et al. (2010); KATHERINE A. MCGLYNN et al. (1995)

GB GIDARO 4/7/2015

Because GSTM1 and GSTT1 catalyze the metabolism of a large number of potentially genotoxic compounds, many studies on the effects of these two genetic polymorphisms have pointed to the formation of DNA adducts and the consequent easier onset of cytogenetic damage (WITH MAJOR PROBLEM IF PRESENT CONTEMPORARILY-and this is not the case for him-and WITH COMMONLY SIGNIFICANT PROBLEMS WHERE PRESENT SINGULARLY,
As in his case.

In particular, the risk of neoplasms of the bladder as well as of the lung is found to be increased, especially with excessive frequency of sausage consumption.

Basic recommendations. Avoid coming into contact with toxic substances both at work and throughout the day. It is also recommended to consume cruciferous vegetables at least twice a week and eat fresh fruits and vegetables regularly to help the body eliminate toxins. The use of detoxifying and purifying supplements can eliminate

Of toxic substances. Glutathione is absolutely, given the genetic framework, indispensable. It must be dosed appropriately, given the altered methylation discussed below.

Relative risks for some cancers for mutations in low-penetrance genes

Tumor site	Gene	Relative risk
Lung	CYP1A1 Exon 7 (Caucasians)	1,30
	CYP2D6	1,26
	GSTM1	1,34
Bladder	NAT-2 "slow"	1,37
	GSTM1	1,57
Colon	NAT-2 "rapid"	1,19

SENSITIVITY TO LIPIDS (FATS) AND GLUCIDS (CARBOHYDRATES or SUGARS).

LIPID and CARDIOVASCULAR METABOLISM.

LPL **C>G CODON 1595**

GENERAL INFORMATION

The lipoprotein lipase (LPL) gene encodes for lipoprotein lipase, an enzyme particularly expressed in heart, muscle, and adipose tissue. Lipoprotein lipase cleaves plasma lipoprotein triglycerides into free fatty acids and glycerol, converting VLDL to LDL, increasing lipoprotein interactions with their receptors located on the cell surface of the vascular endothelium of arteries and capillaries of peripheral tissues. Given the stringent interaction between VLDL production and HDL-C, lipoprotein lipase activity affects plasma cholesterol levels. **Several recent publications report the key role of a variant in the LPL gene (optimal variant) associated with lower levels of triglycerides, LDL, and total cholesterol. The variant is therefore correlated with a lower risk of coronary artery disease.**

GENOTYPIC ASSOCIATION FOUND

GENE	GENOTYPO	RESPONSIVE	PROTECTIVE	RISK
LPL C>G	HET : (G/C) ONE ALLELE CARRIES THE SNP FAVORABLE			!

COMMENT

The genetic variations identified have been associated in the literature with a risk of slightly increased plasma triglycerides and a tendency to have "good" cholesterol (HDL) values

Slightly lower than the population average.

APOC3 C>G POS 3175

GENERAL INFORMATION

Apolipoprotein C3 (APOC3) exerts an important role in lipid metabolism by inhibiting the metabolism of triacylglycerol by the enzyme lipoprotein lipase, resulting in increased triglyceride levels (hypertriglyceridemia). **The T3175G polymorphism of the APOC3 gene is associated with a 4-fold increased risk of hypertriglyceridemia and an elevated risk of occurrence of heart attacks, atherosclerosis, and cardiovascular disease.**

GENOTYPIC ASSOCIATION FOUND

GENE	GENOTYPO	RESPONSIVE	PROTECTIVE	RISK
APOC3 C>G	HET : (C/G) ONE ALLELE CARRIES THE SNP			!

COMMENT

The heterozygous allelic variant was found to be NOT fully physiological, resulting in slight alterations in lipid profile and assimilation relative to the triglyceride and "good" cholesterol (HDL) picture, with easier alteration of the lipidemic profile.

SUGAR METABOLISM AND GLYCATION

Carbohydrate intake results in an increase in blood glucose, which stimulates the release of insulin, a hormone that directs the passage of glucose from the blood into cells. If Insulin is lacking, it either does not work well or there is resistance to its action (also called insulin resistance) and blood glucose levels (blood sugar) tend to rise. Scientific literature suggests that insulin resistance is involved in many diseases including type 2 diabetes, hypertension, and cardiovascular disease. Fasting blood glucose is maintained by the action of hormones within a window of values between 65 and 110 md/dL of plasma. Genetic variations may predispose to increased blood glucose in the presence of excessive consumption of refined sugars. Blood sugar is a very important factor in weight loss. As long as blood glucose is high, insulin prevents the utilization of storage fats and indeed promotes further storage of other fats. The first parameter that is often altered at the blood level is the level of glycated hemoglobin, coming to be the so-called "antechamber" of type 2 diabetes.

Thus, another process that wears the body down is glycation; this is the same process that gives the characteristic color to toast, which causes the pleasant taste of crema catalana or the dry but flavorful appearance of chicken skin in the rotisserie.

Protein glycation is the reaction by which sugars, outside and inside cells, bind to certain groups of proteins to form altered, nonfunctioning molecules called

glycotoxins (AGEs). Glycation products form abnormal bridges between one molecule and another, binding to specific receptors located on the surface of these molecules, and they also accumulate inside cells, thereby carrying out their damaging action. In fact, these glycotoxins bind to tissue proteins, permanently altering or destroying their function.

These glycation products then accumulate in the body and are responsible for many pathophysiological changes. In the long run, they can cause atherosclerosis, renal, vascular, and neurological diseases; in simple words, they accelerate the aging of the body.

As already explained, its genetic condition is significantly associated with both the occurrence of insulin resistance and diabetes.

On the merits:

ANGIOTENSIN CONVERTING ENZYME INS> DEL INTRON 16

GENERAL INFORMATION

Carriers of this genotype also see increased risk of insulin resistance, resulting in hypersensitivity to carbohydrates, ease of weight gain, difficulty with weight loss, and increased susceptibility toward type II diabetes.

GENOTYPIC ASSOCIATION FOUND

GENE	GENOTYPO	RESPONSIVE	PROTECTIVE	RISK
ACE Ins>Del Intron 16	MUT: (Del/Del) BOTH ALLELES CARRY THE UNFAVORABLE SNP			!

COMMENT

The D allelic variant has been determined in the form of the homozygous genotype (D/D), which itself does NOT guarantee physiological enzyme activity. Carriers of this unfavorable variant see increased susceptibility toward forms of insulin resistance.

Of course, we are talking about simple predisposition: it is the interaction between gene and environment a predisposition can then exert its full negative effect.

It is then appropriate to take an overview that takes into account all the genetic variants found and thus also those that follow.

PEROXISOMEN-PROLIFERATOR-AKTIVIERTEN-REZEPTOREN-GAMMA 2 PRO>ALA CODON 12

GENERAL INFORMATION

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor subgroup of the transcription factor family. PPARs form heterodimers with retinoic X receptors, and these heterodimers regulate the transcription of various genes. Three subtypes of PPARs are known, PPAR-alpha, PPAR-delta and PPAR-gamma. Human PPAR-gamma exist in 2 isoforms, gamma-1 and

gamma-2. Yen et al. identified a C to G transversion in the PPARgamma-2 (PPARG2) gene, which results in a Proline (Pro)-Alanine (Ala) substitution on codon 12. **The authors noted that the PPARG2 gene product is a nuclear receptor that regulates adipocyte differentiation and possibly lipid metabolism and insulin sensitivity, i.e., the most important factors in the development of type II diabetes , successful dietary therapy treatment, and body weight control.**

GENOTYPIC ASSOCIATION FOUND

GENE	GENOTYPE	RESPONSIVE	PROTECTIVE	RISK
PPARG2 Pro>Ala codon 12	**WT Pro/Pro** BOTH ALLELES CARRY THE UNFAVORABLE SNP			!

COMMENT

In your case, a genetic variant was found to contribute to the increased genetic predisposition toward alterations in the body's response toward insulin (tendency to manifest insulin resistance over the years) and toward glycation processes.

Insulin resistance and type 2 diabetes are NOT linked to a single genetic mutation, but require an overview that examines polygenic variants. Fundamental, however, is recovering motor activity.

To counteract metabolically unfavorable conditions, regaining motor activity is crucial, and eating whole foods is preferable.

Of course, we are talking about simple predisposition: it is the interaction between gene and environment that a predisposition can then exert its full negative effect.

In this context, however, try to prioritize the intake of foods with the lowest glycemic index, while still not exaggerating in the glycemic load of your food day.

Brief bibliographical citation

Zhang R et al. ***Effects of Pro12Ala polymorphism in peroxisome proliferator-activated receptor-γ2 gene on metabolic syndrome risk: a meta-analysis.*** *Gene. 2014 Feb 1;535(1):79-87.*

Buzzetti R et al. ***The common PPAR-gamma2 Pro12Ala variant is associated with greater insulin sensitivity.*** *Eur J Hum Genet. 2004 Dec;12(12):1050-4.*

Dongxia L et al. ***Association of peroxisome proliferator-activated receptorgamma gene Pro12Ala and C161T polymorphisms with metabolic syndrome.*** *Circ J. 2008 Apr;72(4):551-7.*

CEREBROVASCULAR SUSCEPTIBILITY-METHYLATION PROCESS

METHYLENETETRAHYDROFOLATE REDUCTASE ALA>VAL CODON 222

GENERAL INFORMATION

The enzyme methylenetetrahydrofolate reductase (MTHFR) is involved in **folate metabolism**. It catalyzes the transformation of 5-methyltetrafolate from 5,10-methylenetetrahydrofolate. Folate is a cofactor for homocysteine remethylation; without such remethylation, plasma homocysteine levels increase. Elevated plasma levels of homocysteine **are a risk factor for cardio and cerebrovascular disease** and may be caused by genetic variations in MTHFR. A common polymorphism in the MTHFR gene (C677T, Ala222Val) is associated with **decreased activity of the enzyme, leading nonsevere forms of hyperhomocysteinemia**; in fact, the substitution of a **C** (*cytosine*) for **T** (*thymine*) at nucleotide **677** (**C677T**) causes a substitution of an *alanine* for *valine* in the final protein and a **reduction in MTHFR enzyme activity of 50/70% in homozygous individuals and about 35% in heterozygous individuals**. This variant results in elevated blood levels of homocysteine **especially after oral methionine loading**. Increased **levels of homocysteine** in the blood are now considered **a risk factor for vascular disease**, (arterial thrombosis/stroke), probably through a mechanism mediated by sulfhydryl groups on the endothelial wall of vessels. Double heterozygosity conditions, especially with the Leiden variant of factor V carries or the 20210 variant of prothrombin, may increase the relative risk for venous thromboembolism.

GENOTYPIC ASSOCIATION FOUND

GENE	GENOTYPO	RESPONSIVE	PROTECTIVE	RISK
MTHFR Ala>Val Codon 222	**WT : (Wing/Ala)** NO ALLELE CARRIES SNP		**okay**	

COMMENT

The favorable physiological allelic variant was detected, which does NOT negatively affect folate and vitamin B12 metabolism, NOT constitutionally favoring increased blood homocysteine levels. METILATION PROCESS in the normal range with regard to this gene.

VITAMIN D METABOLISM

VITAMIN D RECEPTOR	Taq 1 - C>T

GENERAL INFORMATION

The Vitamin D receptor mediates the effects of Vitamin D on bone metabolism and regular cell division. A snip of this gene can significantly increase the risk of osteoporosis,

but also the risk of cancer, especially bowel and prostate cancer. These risks can be minimized by proper nutrition or the use of specific dietary supplements.

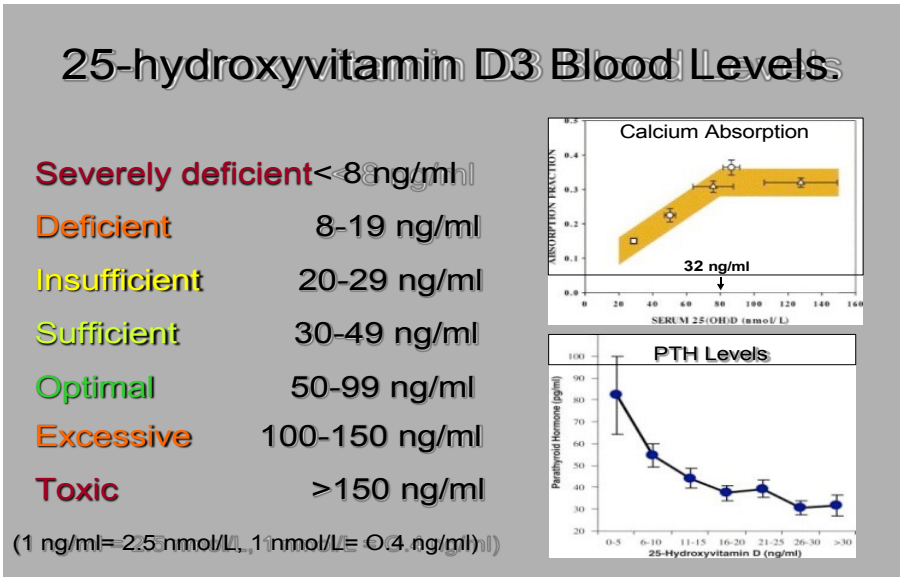
GENOTYPIC ASSOCIATION FOUND

GENE	GENOTYPE	RESPONSIVE	PROTECTIVE	RISK
VDR	HET (C/T) ONE ALLELE CARRIES THE SNP			!

COMMENT

The allelic versions of the genes analyzed have been associated by the literature with decreased calcium absorption and ossification, finding NOT fully physiological (heterozygous alteration) vitamin D receptor. This results in an increased susceptibility toward osteopenia.

Vitamin D plays a very important role of factual stimulation on the immune system, so it is important to maintain adequate blood levels of it. Carriers of this genotype need optimal levels, as the receptor is as if it cannot interact effectively with vitamin D.



MICROBIOT TEST - commentary

- ✓ **Altered Bacteroides/Firmicutes ratio,** with relative excess of the former. Among the **Bacteroides** good presence of Bacteroides fragilis and sub-deficiency of Prevotella, with **pro-inflammatory significance.**

- ✓ **Altered commensal flora.** Total commensal abundance is a sum total of reported commensal bacteria compared to a healthy cohort; higher total commensal abundance, as in your case, **indicates overgrowth of pathogenic bacteria. Not only does there exist a, albeit contented, overgrowth of the Hafnia species.** Specifically:

- **Mild sub-deficiency of both Bifidi and Lactobacilli.**
- **Absence of transient pathogenic bacterial flora.**
- **Absence of overgrowth of fungal species.**
- **Presence of overgrowth of saprophytic bacterial species, such as Citrobacter (the latter in pathogenic amounts), Serratia and Klebisella.**
- **Mild overgrowth of Hafnia species. On the merits: mild/moderate infestation by Hafnia species, with risk of contained histamine mackerel syndrome, which can also be associated with the reported complaints.** Hafnia alvei base is commensal flora, which, however, in his case is present in pathogenic amounts and in this creates local and systemic problems, as well as problems in the urinary tract (example: cystitis). This picture can be likened to a **clinical picture of excessive histamine release, also called "mackerel-toxin."** **Mackhombrotoxin** refers to a mixture of toxins containing mainly histamine and other histaminesimilar molecules (putrescine and cadaverine), which can sometimes be found in high concentrations in some **fish, shellfish, and crustaceans** and which, due to the activity of certain enzymes (histidine decarboxylase) convert **histidine** to histamine. The rate of conversion from histidine to histamine is not identical for all bacterial species and depends greatly not only on the type of bacterial flora but also on the storage conditions of fish products (temperature, humidity, partial pressure of oxygen). Studies have shown that some species, such as ***Hafnia alvei***, are capable of producing large amounts of histamine; if the contaminated product is consumed, symptoms of intoxication occur. Cooking, smoking and storage by canning do not eliminate the toxin produced. The minimum temperature for histamine formation by the producing bacteria is about 0°C, while the optimal range is between 0°C and 10°C. Associated **symptoms**: among the most common are skin rash, especially on the face and neck. Also frequent are nausea and vomiting, diarrhea, and abdominal cramps. Toxicity depends on many factors. If the storage conditions of the fish product are poor, other secondary amines such as cadaverine (derived from ornithine) and putrescine (derived from lysine) are also formed. Both are not very toxic as such, but they indirectly enhance histamine metabolism through their action on DAO (deamino oxidase) **and HMT** (N-methyltransferase) enzymes, in other words, they inhibit histamine degradation by these enzymes. There are three receptors to which histamine binds and they are called H1, H2 and H3 and each mediates different effects. In addition to the symptoms mentioned before, the H1 receptor mediates arrhythmogenic effects, while H2 increases ventricular and sinus automatism. The most frequent reactions that occur in the body following the ingestion of a food with high histamine content (> 200 to > 500 mg/100 g) are:

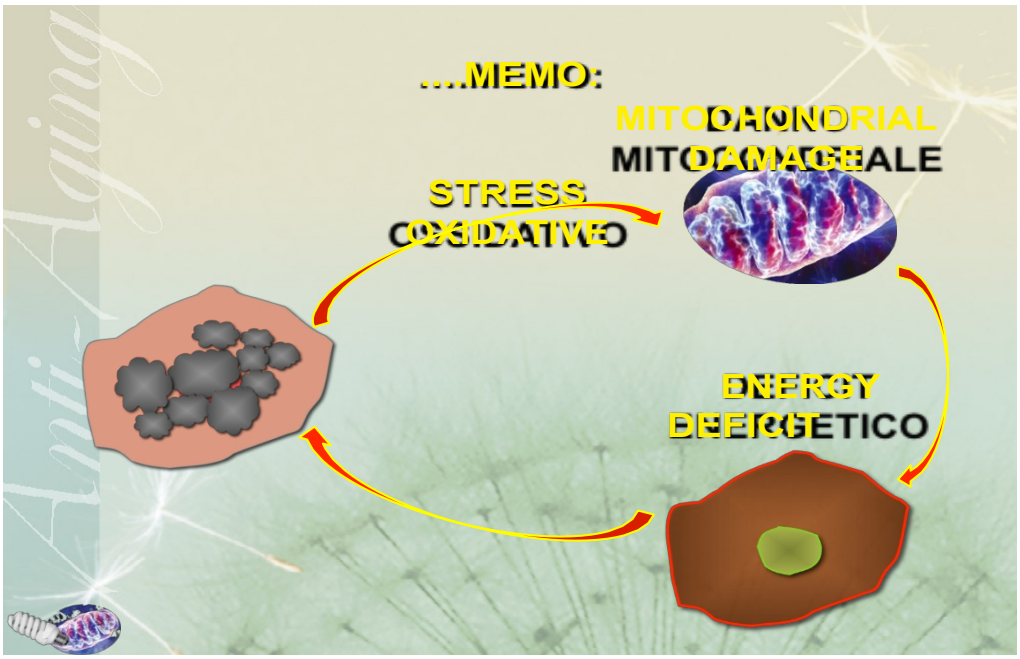
- In *oral allergic syndrome*, symptoms mainly affect the oral cavity. They are represented by stinging itching in the oropharynx, appearance of papules-vesicles in the mucosa, and edema of the lips. Urticaria, diarrhea, and vomiting may also occur if the food is ingested;
- *Gastroenteritic issues*. Again if the food is ingested in contact with the intestinal mucosa, symptoms may give diarrhea, vomiting or systemic reactions, such as, hives or similar reactions;
- *urticarial-angioedema syndrome*. Urticaria and angioedema are among the most common symptoms in food reactions, especially responsible are fish and shellfish;
- *Respiratory manifestations*: rhinitis, conjunctivitis, sinusitis and serous otitis media, bronchial asthma.
- *Systemic manifestations*: exacerbation of exhausted conditions, and inflammation.
- *APPROPRIATE TO REDUCE THE INTAKE OF HISTAMINE-RELEASING FOODS*.

BLOOD EXAMINATIONS - commentary

- **Folic acid: high BUT NOT a problem. Simply unnecessary supplementation of it, given also the genetic picture.**
- **Vitamin B12: sub-deficient, its supplementation useful but not as cyanocobalamin.**
- **Vitamin C: marked deficiency. Fundamental to its supplementation.**
- **Vitamin D and Vitamin E: optimal values.**
- **Interleukin profile: especially high levels of interleukin-1 and TNF-alpha, which contribute strongly to the inflammatory picture and are often associated with abnormal immunologic reactivity. I believe they underlie her pathological/symptomatic picture.**
- **Omega screening, relatively in the normal range. There is almost an excess of omega-3 of the EPA class, cwhich may lead to a biosynthetic increase in radical species. In parallel, there is a sub-shortage of omega-3 of the DHA class, which needs to be selectively supplemented.**
- **Extremely elevated PCR. It not only indicates an elevated inflammatory condition, but also is associated with an infectious status that needs to be investigated further, also in light of the altered immune test showing positivity for diagnosis of SYSTEMIC LUPUS.**

- **<u>Viral markers</u>: not significantly altered.**

- **<u>Coenzyme Q10</u>: severe deficiency with effect of mitochondrial dysfunctionality (see image), leading to lower metabolic energy (interference in ATP formation) and easier pro-oxidative alteration (more free radicals and higher nitrosative stress).**



❖ **<u>Alteration of liver and muscle distress parameters (transaminases and CPK)</u>. Fundamental to activate detox therapy, which can give concrete results especially if by intravenous infusion. The alteration of CPKs associated with that of coenzyme Q10 will require further investigation for possible mitochondrial pathology. All this also at the luc e of metabolomics examination showing excess acylcarnitine, with Krebs cycle/mitochondrial dysfunctionality. Peak-related alterations in some of the acetylcarnitine isoforms also depose disturbances in fatty acid transport and mitochondrial oxidation. One of the pathogenic mechanisms of CFS (chronic fatigue syndrome) is dysregulation of mitochondria metabolism resulting in a deficit in ATP production and impaired ion transport. This dysregulation is promoted by the presence of xenobiotic substances that bind to the active sites of Translocator Protein (TL) and alter both the transport of ATP, produced in the mitochondrion, and the transport of cytosolic ADP. This results in reduced ATP outflow from the mitochondrion to the cytosol and reduced ADP entry from the cytosol to the mitochondrion. Reduced transport of energy (ATP) to the cytosol, consequently associated with reduced mitochondrial ATP production, promotes the development of the main clinical picture of Chronic Fatigue characterized by fatigue, associated with asthenia, fatigability, and lassitude. All of which is also promoted by alterations in the carnitine cycle and fatty acid beta-oxidation, as found in his case. Theoretical suspicion of alteration of the metabolic pathway leading to the excessive formation of peroxynitrite, which can then attack various components of the mitochondrion, causing a deficiency of**

NAD/NADH reserves and thus consequently reduced ATP. In additionnitric oxide inhibits cytochrome oxidase activity, also causing mitochondrial dysfunction.

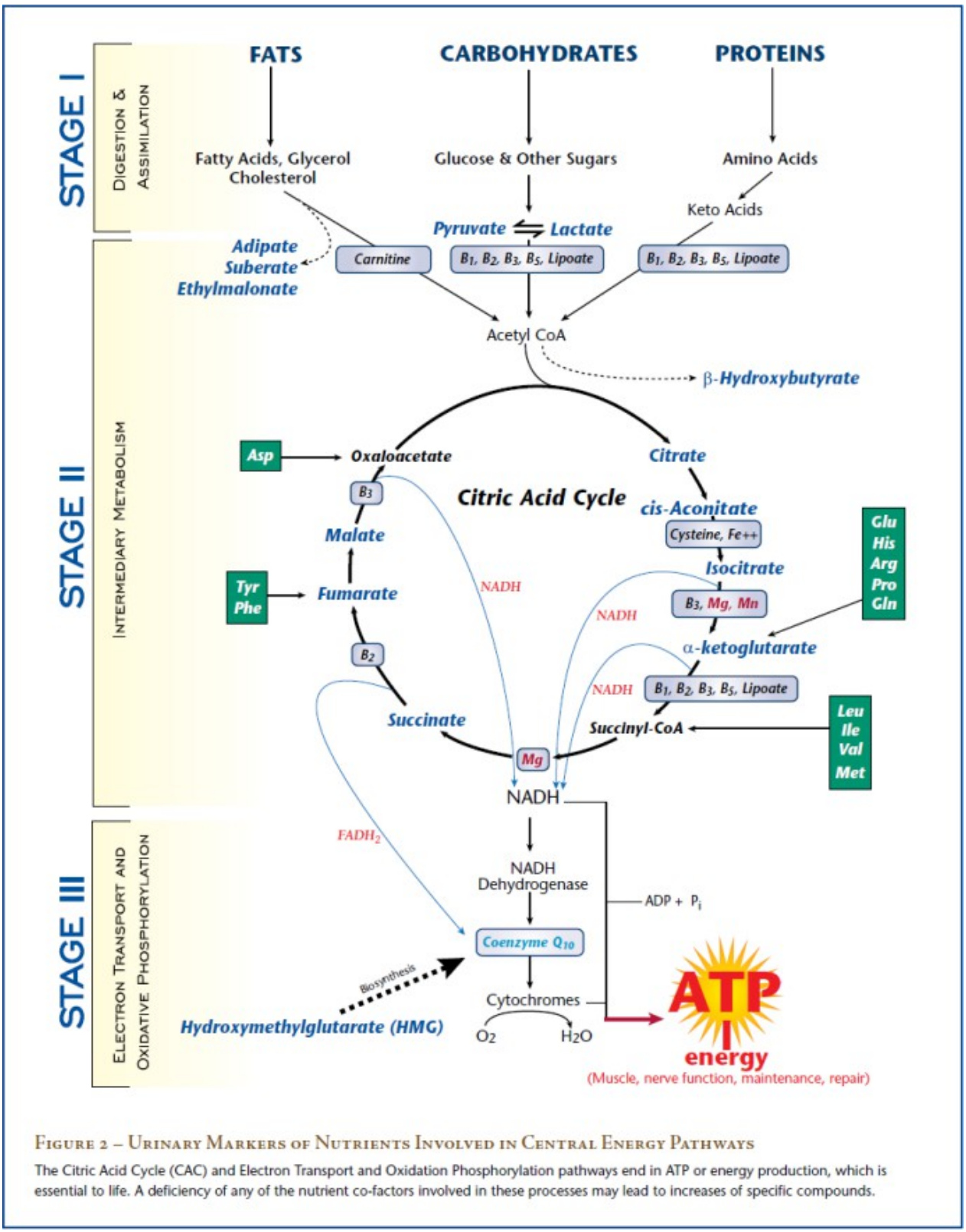


FIGURE 2 – URINARY MARKERS OF NUTRIENTS INVOLVED IN CENTRAL ENERGY PATHWAYS

The Citric Acid Cycle (CAC) and Electron Transport and Oxidation Phosphorylation pathways end in ATP or energy production, which is essential to life. A deficiency of any of the nutrient co-factors involved in these processes may lead to increases of specific compounds.

Prof a c dr Damiano Galimberti