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Genes, Diet and Uric Acid Nephrolithiasis

Uric acid represent the final product of purine metabolism: one-third of daily uric acid production is excreted by the gastrointestinal tract and two-thirds by the kidney. A high uric acid excretion with urine, a low urine volume due to dehydration and an acidic urinary pH value have been suggested to be the most important risk factor for uric acid nephrolithiasis (UAN). Recently mutation analysis showed that a variant (Ala62Thr) in a specific protein isoform (Talanin) is associated with UAN. We found that this variant is rather common in the Sardinian (32%) and Sicilian populations (23%), that are Mediterranean islands, as well as in the Italian peninsula (27%). On the contrary, in Burkina Faso and in Benin, both sub-Saharan countries, mesoendemic regions for Plasmodium falciparum malaria and other parasite infections, a low incidence of this variant was found (1.1% and 1.2% respectively). In Burkina Faso and in Benin, the low incidence of Ala62Thr variant is associated with low presence of UAN and the major classes of stones reported are calcium oxalate and calcium phosphate. These low frequencies for Ala62Thr predisposing to UAN in Burkina Faso and in Benin may represent the result of a selective mechanism where the arid conditions of territory and the characteristic alimentary habits of this part of Africa may represent an obstacle to the expansion of mutated allele.

Keywords: uric acid nephrolithiasis, urate oxidase, *ZNF365* gene, nutrition

Clinical aspects of Uric Acid Nephrolithiasis

Kidney stones are estimated to affect 10% of the population (Serio and Fraioli 1999; Rivers et al. 2000). The major classes of formed stones are calcium oxalate, calcium phosphate and uric acid. Uric acid nephrolithiasis [MIM 605990] accounts for 20% of all stones and it ensues when the urine becomes overly concentrated with uric acid that may form small crystals and, subsequently, stones (Jaeger 1996; Curhan et al. 1997). Every year a considerable number of people are hospitalized for this disease. Thus, understanding the genetic factors that contribute to the development of this disorder will lead to improved diagnosis, treatment and prevention, and will also contri-

bute to stem or even to reverse the predicted rise in prevalence of this disease. Uric acid represent the final product of purine metabolism: one-third of daily uric acid production is excreted by the gastrointestinal tract and two-thirds by the kidney (Roch-Ramel, 1999). Hyperuricemia and consequently hyperuricosuria occur in the presence of excessive dietary consumption of purines from meat and fish or when there is altered urate elimination (Siener and Hesse 2000). A high uric acid excretion with urine, a low urine volume due to dehydration and an acidic urinary pH value have been suggested to be the most important risk factor for urine uric acid stone formation. The clustering of uric acid nephrolithiasis in restricted family groups suggests the existence of a predisposing set of genes.

Genetical basis of Uric Acid Nephrolithiasis

Recently, we identified a susceptibility locus for human Uric Acid Nephrolithiasis (UAN) on 10q21-q22 and demonstrated that a novel gene (*ZNF365*) included in this region produces through alternative splicing, 4 different transcripts: *A*, *B*, *C* and *D* coding for different protein isoforms. Mutation analysis showed that a variant (Ala62Thr) in a specific protein isoform (Talanin) of this gene is associated with the disease (Ombra et al. 2001; Gianfrancesco et al. 2003). By evolutionary analysis, it seems that transcript *ZNF365D*, encoding talanin protein isoform predisposing to UAN, emerged during primate evolution from a non-coding genomic sequence. This evolved in a standard gene structure and assumed its role in parallel with the disappearance of uricase, probably against a disadvantageous excessive hyperuricaemia (Gianfrancesco et al. 2004). Since the born of a novel gene and its geographic distribution may be influenced by environmental factors, we analyzed the frequency of this polymorphism (Ala62Thr) in different ethnic groups from the Mediterranean and African areas with different environmental and alimentary characteristics (Figure 1). We found that this variant is rather common in the Sardinian (32%) and Sicilian populations (23%), that are Mediterranean islands, as well as in the Italian peninsula (27%). On the contrary, in Burkina Faso and in Benin, both sub-Saharan countries, mesoendemic regions for *Plasmodium falciparum* malaria and other parasite infections, a low incidence of this variant predisposing to UAN was found (1.1% and 1.2% respectively) (Gianfrancesco et al. 2004). This low frequency for Ala62Thr predisposing to UAN in Burkina Faso and in Benin could be represent the result of a selective mechanism where the arid conditions of territory may represent an obstacle to the expansion of mutated allele. This result suggests also that the characteristic alimentary habits of African people living

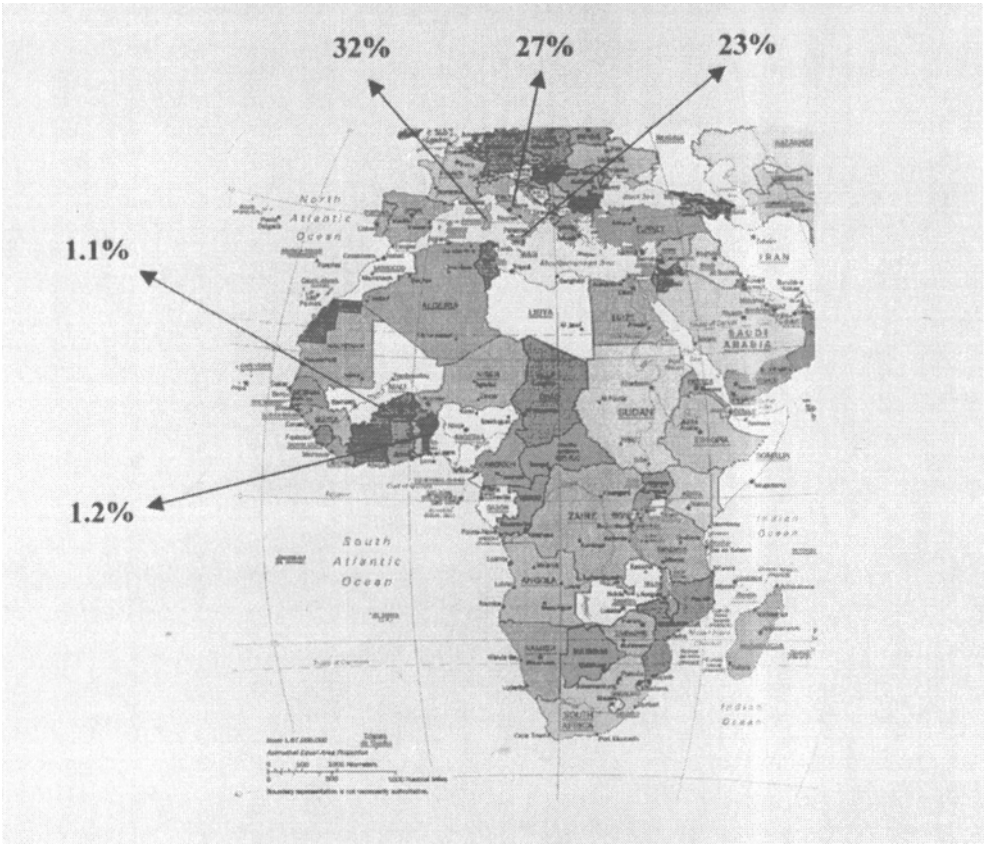

Figure 1. Frequency of Ala62Thr variant in different ethnic groups from the Mediterranean to African areas with different environmental and alimentary characteristics.

in Burkina Faso and in Benin may have impeded the diffusion of this polymorphism. Our investigation about the incidence of uric acid nephrolithiasis among black population confirmed that this was absolutely rare in this part of Africa, where the oxalic and phosphate nephrolithiasis is prevalent, while in Western countries including Sardinia and Sicily, the incidence of uric acid nephrolithiasis is frequently represented (20% of all stones).

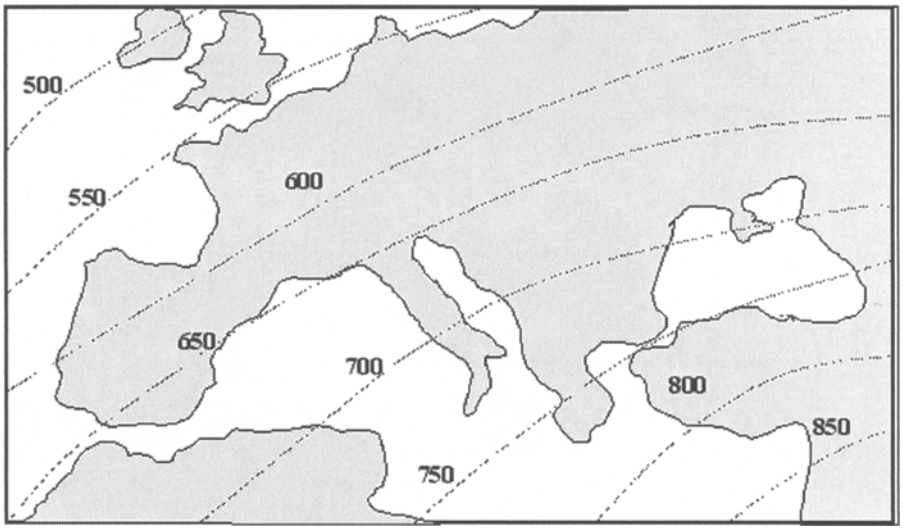

Figure 2. *South-East North-West progression of agriculture in the recent prehistory (from 8500 years BP to 5000 years BP) (From Chiarelli B. 2003).*

The evolutionary adaption

In some mammals uric acid that is produced during the metabolism of purines is degraded by the hepatic enzyme "uricase" (urate oxidase) to allantoin, and is excreted with urine. During the Miocene epoch the gene encoding for uricase mutated, determining a progressive enzyme activity disappearance both in humans and great apes (Wu et al. 1992). As a consequence of this mutation, in these species the uric acid is excreted unmodified with urine as final product of purine metabolism. The mutation of the urate oxidase gene could be considered an evolutionary advantage for the elevated blood uric acid levels (Christen et al. 1970; Wacker 1970). In fact, some evidence, suggest that high concentration of uric acid, especially in human blood, provided an advantage because uric acid has a role as antioxidant, being a powerful scavenger of free radicals, and it could have been involved in the mechanism of longevity (Ames et al. 1981; Whiteman and Halliwell, 1996). Other evidences suggest that an increased level of uric acid in blood maintains blood pressure under low-salt dietary conditions, providing a survival advantage (Watanabe et al. 2002). However currently in western countries, hyperuricemia is associated with the risk of hypertension and of cardiovascular and renal diseases (Selby et al. 1990, Jossa et al. 1994, Wu et al. 1992, Cannon et al.

1966, Alderman et al. 1999, Verdecchia et al. 2000, Johnson et al. 2003). Probably the development of an homeostatic system for blood/renal urate exchange against an excessive and detrimental level of uric acid in blood, should be advantageous. This homeostatic system which results from the loss of uricase and consequently from excessive presence of uric acid in blood, could be influenced by environmental conditions, alimentary habits and anthropological and socio cultural characteristics of the region.

Influence of alimentary habits

The low frequency of Ala62Thr allele in Burkina Faso and in Benin was not surprising if we consider that it could be the result of a selective mechanism, where more arid conditions of the territory, could have represented an obstacle to the expansion of mutated allele. Moreover, anthropological considerations suggest that also the alimentary habits of ancient Africans could have selected the wild-type allele. In the past, the meat consumption was prevailing on the vegetable and fruit consumption (Larsen 2003; Nestle 1999). Meat in particular was acquired opportunistically via hunting of small or young animals and scavenging of animals killed by other species. At some point of prehistory, humans began to hunt cooperatively, making possible the acquisition of meat from large game. These alimentary characteristics of homo sapiens should facilitate the maintenance of a wild-type gene (Ala62), which facilitate the elimination of uric acid with urine, while the variant gene (Thr62) allele was disadvantaged because it causes kidney damage through UAN (Kang et al 2002). The study of archaeological human remains of approximately 10,000 years ago, from around the world reveals that this period in human dietary history saw a decline in health, including increased evidence of morbidity (poorer dental health, increased occlusion abnormalities, increased iron deficiency anaemia, increased infection and bone loss).

After that period there was around to the Mediterranean sea the agriculture diffusion (Chiarelli, 2003), that produced many changes but also the appearance of some polymorphisms more adapted at the new realities (Figure 2). The hypothesis that a prevalently carnivorous alimentation could have a selective disadvantage for the Ala62Thr allele is sustained also from the observation that the frequency of type 2 diabetes due to insulin resistance in African population living at south of Sahara is prevalent (70-90%) respect the type 1 diabetes (Sobngwi et al 2001; Elbagir et al 1998; Ducorps et 1997). This observation suggests a genetic component in type 2 diabetes, which was selected by the low intake of carbohydrates with the diet because a prevalent carnivorous alimentation. In fact, Miller and Calagiuri (1994) have suggested that low car-

bohydrate carnivorous diet could have disadvantaged reproduction in insulin sensitive individuals and advantaged it in individuals with insulin resistance. If we consider that modern *homo sapiens* is believed to originate from Africa some time between 100-200 thousand years ago and subsequently migrated throughout the world, European populations must have naturally a lower frequency of insulin resistance because they were among the first to adopt agriculture and their diet has been high in carbohydrates for 10,000 years. Then the disadvantage of insulin resistance gene (i.e. a low carbohydrate diet) and the advantage of Thr62 allele could be therefore relaxed much sooner in European population, which was exposed to high carbohydrate/protein alimentary ratio for a sufficient long period of time. This hypothesis is further sustained by the observation that both genetic and environmental factors play a critical role in the development of type 2 diabetes both in developing countries (Rotini et al 2004) and in African Americans, where an excess body weight due to increased caloric intake and sedentary life style have been recognized to be the major risk factors (Zimmet et al 2001).

The favourable environmental conditions of Mediterranean areas and the alimentary habits of so called Mediterranean diet pyramid could have favoured the expansion of Ala62Thr mutant allele. The Mediterranean diet pyramid is based on food patterns typical of Greece and southern Italy, where adult life expectancy was among the highest in the world and rates of coronary heart disease, certain cancers, type 2 diabetes and other diet-related chronic diseases were among the lowest (Siener and Hesse 2000; Ulijaszek 2002) .

The different frequency of mutant Ala62Thr allele in Sardinia (31%) and in Sicily (23%) could also be explained by the different geographic position and by the different socio cultural characteristics of these two islands. Sardinia could be considered a genetic isolate (Arcos-Burgos and Muenke M. 2002) differently from the Sicily, where the presence of several genetic anomalies (Hb S, HbC, variant of beta Thalassemia, metabolic defects) coming from all part of Europe, Africa and Asia, must be considered as a pot where different genes are brewing (Schiliro 1978; Schiliro et al 1997). On the other hand in Burkina Faso and in Benin, the low incidence of Ala62Thr variant is associated with low presence of UAN. In this part of Africa the major classes of stones reported are calcium oxalate and calcium phosphate (Balla et al 1998; Robertson 2003). In the tropics, the stone problem is compounded by low urine volumes resulting in some areas from poor drinking water, from chronic diarrhea, and in others from the hot climate and fluid losses through the sweat. As nutrition improves in African countries, the formation of bladder stones gives way to upper urinary tract stones consisting of calcium oxalate, often mixed with calcium phosphate or uric acid, such as are formed in most Western countries. The effect of environmental on the genetic structure of human will open the way to the identification of new genes for multifactorial human diseases.

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References

Alderman MH, Cohen H, Madhavean S, Kivlighn S (1999) Serum uric acid and cardiovascular events in successfully treated hypertensive patients. *Hypertension* 34:144-150

Ames BN, Cathcart R, Schwiers E, Hochstein P (1981) Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. *Proc Natl Acad Sci U S A* 78:6858-6862.

Arcos-Burgos M, Muenke M (2002) Genetics of population isolates. *Clin Genet* 61:233-247.

Balla AA, Salah AM, Khattab AH, Kambal A, Bongartz D, Hoppe B, Hesse A (1998) Mineral composition of renal stones from the Sudan. *Urol Int* 61:154-156.

Cannon PJ, Stason WB, Demartini FE, Sommers SC, Laragh JH (1966) Hyperuricemia in primary and renal hypertension. *N Engl J Med* 275:457-464

Chiarelli B (2003) *Dalla Natura alla Cultura* Vol.II. Piccin-Nuova Libraria ed. Padova

Christen P, Peackock WC, Christen AE, Wacker WE (1970) Urate oxidase in primate phylogenesis. *Eur J Biochem* 12:3-5

Curhan GC, Willett WC, Rimm EB, Stampfer MJ (1997) Family history and risk of kidney stones. *J Am Soc Nephrol* 8:1568-1573

Ducorps M, Ndong W, Jupkwo B, Belmejdoub G, Poirier JM, Mayaudon H, Bauduceau B, (1997) Epidemiological aspects of diabetes in Cameroon: what is the role of tropical diabetes? *Diabetes Metab* 23: 61-67

Elbagir MN, Eltom MA, Berne C (1998) Classification of newly-onset IDDIM and NIDDIM in subjects aged 25-45 years in Sudan. *J Intern Med* 244, 443-450

Gianfrancesco F, Esposito T, Ombra MN, Forabosco P, Maninchedda G, Fattorini M, Casula S, Vaccargiu S, Casu G, Cardia F, Deiana I, Melis P, Falchi M, Pirastu M (2003) Identification of a novel gene and a common variant associated with uric acid nephrolithiasis in a Sardinian genetic isolate. *Am J Hum Genet* 72:1479-1491

Gianfrancesco F, Esposito T, Casu G, Maninchedda G, Roberto R, Pirastu M. (2004a) Emergence of Talanin protein associated with human uric acid nephrolithiasis in the hominidae lineage 2004, Gene: 339:131-138.

Gianfrancesco F, Esposito T, Piras D, Casu G., Musumeci S (2004b) Geographic distribution of Ala62Thr variant associated to Uric Acid Nephrolithiasis in Sub-Saharan and Mediterranean area. *Int.J Anthop.* (this issue).

Jaeger P (1996) Genetic versus environmental factors in renal stone disease. *Curr Opin Nephrol Hypertens* 5:342-346

Johnson RJ, Kang DH, Feig D, Kivlighn S, Kanellis J, Watanabe S, Tuttle KR, Rodriguez-Iturbe B, Herrera-Acosta J, Mazzali M (2003) Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? *Hypertension* 41:1183-1190

Jossa F, Farinaro E, Panico S, Krogh V, Celentano E, Galasso R, Mancini M, Trevisan M (1994) Serum uric acid and hypertension: the Olivetti heart study. *J Hum Hypertens* 8:677-681

Kang DH, Nakagawa T, Feng L, Watanabe S, Han L, Mazzali M, Truong L, Harris R, Johnson RJ (2002) A role for uric acid in the progression of renal disease. *J Am Soc Nephrol* 13:2888-2897.

Larsen CS. (2003) Animal source foods and human health during evolution. *J Nutr* 133:3893S-3897S.

Miller JC, Colagiuri S (1994) The carnivore connection: dietary carbohydrate in the evolution of NIDDM. *Diabetologia* 37:1280-6.

Nestle M. (1999) Animal v. plant foods in human diets and health: is the historical record unequivocal? *Proc Nutr Soc* 58:211-218.

Ombra, M.N., Forabosco P, Casula S, Angius A, Maestrale G, Petretto E, Casu G, Colussi G, Usai E, Melis P., Pirastu M. (2001). Identification of a new candidate locus for uric acid nephrolithiasis. *Am J Hum Genet* 68:1119-1129.

Rivers K, Shetty S, Menon M (2000) When and how to evaluate a patient with nephrolithiasis. *Urol Clin North Am* 27:203-213.

Rotimi CN, Chen G, Adeyemo AA, Furbet-Harris P, Guass D. Zhou J, Berg K, Adegoke O, Amoah A, Owusu S et al (2004) A genome-Wide search for type 2 diabetes susceptibility genes in West Africans. *Diabetes* 53: 838-841.

Robertson WG (2003) . Renal stones in the tropics. *Semin Nephrol* 23:77-87.

Roch-Ramel F, Guisan B (1999) Renal transport of urate in humans. *News Physiol Sci* 14:80-84

Schiliro G (1978) Sicily: the world reservoir for thalassemias and haemoglobinopathies. *Nature* 276: 761.

Schiliro G, Mirabile E, Testa R, Russo-Mancuso G, Dibenedetto SP, (1997) Presence of hemoglobinopathies in Sicily: a historic perspective. *Am J Med Genet.* 69:200-206.

Selby JV, Friedman GD, Quesenberry CP (1990) Precursors of essential hypertension: pulmonary function, heart rate, uric acid, serum cholesterol, and other serum chemistries. *Am J Epidemiol* 131:1017-1027.

Serio A, Fraioli A (1999) Epidemiology of nephrolithiasis. *Nephron Suppl* 81:26-30.

Siener R, Hesse A. (2003) The effect of a vegetarian and different omnivorous diets on urinary risk factors for uric acid stone formation. *Eur J Nutr* 42:332-337.

Sobngwi E, Mauvais-Jarvis F, Vexiau P, Mbanya JC, Gautier JF, (2001) Diabetes in Africans. Part 1: Epidemiology and clinical specificities. *Diabetes Metab* 27: 628-634.

Ulijaszek SJ (2002) Human eating behaviour in an evolutionary ecological context. *Proc Nutr Soc* 61:517-526.

Verdecchia P, Schillaci G, Reboldi GP, Santeusanio F, Porcellati C, Brunetti P (2000) Relation between serum uric acid and risk of cardiovascular disease in essential hypertension. The PIUMA study. *Hypertension* 36:1072-1078

Wacker WE (1970) Man: sapient but gouty. *N Engl J Med* 283:151-152

Watanabe S, Kang DH, Feng L, Nakagawa T, Kanellis J, Lan H, Mazzali M, Johnson RJ, (2002). Uric acid, hominoid evolution, and the pathogenesis of salt-sensitivity. *Hypertension* 40:355-360

Whiteman M, Halliwell B (1996). Protection against peroxynitrite-dependent tyrosine nitration and alpha 1-antiproteinase inactivation by ascorbic acid. A comparison with other biological antioxidants. *Free Radic Res* 25:275-283

Wu X, Muzny DM, Lee CC, Caskey CT (1992) Two independent mutational events in the loss of urate oxidase. *J Mol Evol* 34:78-84.

Zimmet P, Alberti KG, Shaw J (2001) Global and societal implications of the diabetes epidemic. *Nature* 414 :782-787.

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