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Studies of bone mineral density in children affected by dietary intolerances

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PP4-The Pediatric Department of the University of Ferrara

Abstract — The Pediatric Department of the University of Ferrara has developed a special expertise in the field of hemoglobinopathies and has also an interest in gastrointestinal diseases. It has a long standing collaboration with the Department of Genetics of the University of Verona. The main fields of interest are thalassemia, gluten intolerance, and osteoporosis in its various aspects. Using our previous research experience as a platform, we plan to study Vitamin D metabolism, bone mineral density, the FGF23 and Klotho axis in patients with thalassemia, in patients with adult type lactose intolerance, in patients treated with antiepileptic drugs and in those who suffer from gluten intolerance. Finally, we intend to cooperate with another group (PP9) of the Trans2Care project in order to clarify the role of tissue antitransglutaminases in seronegative patients with symptoms of gluten intolerance.

Index Terms — FGF23/Klotho, lactose intolerance, Bone metabolism, Thalassemia, gluten intolerance, pseudoxanthoma elasticum

1 INTRODUCTION

The University of Ferrara was founded by Alfonso I d'Este in 1321. It has been the Alma Mater to Paracelsus, Nikolaus Kopernikus, and Pico della Mirandola. Today, the Department of Pediatrics of the University of Ferrara is situated in the Sant'Anna Hospital in the city center centre. but It will be moved soon to a new facility 10 Km to the south where larger and newer spaces will be available. The staff comprises physicians, administrative personnel, one dietitian, and, with the support of Trans2Care, a new collaborator has just been enrolled. The Department has a

special expertise in the field of Thalassemia and other hemoglobinopathies, and in neurological disorders of childhood. In addition, part of our staff has an interest in gastrointestinal diseases.

2 ONGOING RESEARCH

2.1 Multicentre Study of Survival in Thalassemia major.

The team manager of PP4 has, for the past three decades, coordinated a cooperative study on survival, complications and diagnostic and therapeutic aspects of thalassemia. The results of the study on survival and complications have been regularly published [1], [2], [3], [4]. The most recent data, 20 years from the first collection, show that the prognosis of these patients has greatly improved. In fact, our results show a sharp decrease in mortality in the last few years, testifying to the efficacy of modern therapy. i.e. MRI for the quantification of iron in the heart [5] and liver, the oral iron chelators [6], [7], aggressive management of cardiac and hepatic complications.

2.2 Feasibility of a screening for hemoglobinopathies

In order to identify early the infants affected by hemogolobinopathies, in particular sickle cell anemia, we are collecting the cord blood from all the neonates who are born in Ferrara. In fact an early diagnosis of sickle cell anemia can prevent infection and death in the first years of life [8]. In the past year, 1600 samples were examined by HPLC and 19 were found to be abnormal.

2.3 Morbidity and Mortality according to sex

It is well documented that, female patients with thalassemia major survive longer than males [9]. In order to clarify the origin of this phenomenon, we have measured by magnetic resonance imaging the iron concentration in the heart of 776 patients (370 males) and have compared the results obtained in males and females, with and without cardiac failure or arrhythmias. As expected, cardiac disease was higher in males than in females, but no difference in cardiac iron content was observed according to sex [10]. Therefore, we concluded that males and females are at the same risk of accumulating iron in their hearts but females seem to tolerate iron toxicity better, possibly as an effect of reduced sensitivity to chronic oxidative stress. This study was conducted in collaboration with the Myocardial Iron Network in Thalassemia.

2.4 Vitamin D and Osteopenia in Thalassemia

Osteoporosis and osteopenia are frequent complications of thalassemia major and intermedia. In a cooperative study, [11] we found osteoporosis to be present in the great majority of patients with thalassemia intermedia and in 115/239 patients with thalassemia major. In thalassemia major, no association was found with

specific polymorphisms in candidate genes (vitamin D receptor, estrogen receptor, calcitonin receptor, and collagen type 1 alpha 1). Osteoporosis in female patients with thalassemia major was strongly associated with primary amenorrhea, while in male patients, hypogonadism was not significantly related to bone mineral density. Low bone mineral density was also associated with cardiomiopathy, diabetes mellitus, chronic hepatitis, and increased ALT.

2.5 Genetic of lactose intolerance

We studied the presence of a substitution of C to T- single nucleotide polymorphism (rs4988235, -13910C>T), at position -13910 bp upstream in the lactose gene (rs4988235), in a population of Italian children and their parents. The children were diagnosed as lactose intolerant on the basis of the breath hydrogen test [12]. The mutation, localized in a regulatory region, was found to be strongly associated with the lactase persistence phenotype in North-European population. We confirmed its presence in Southern Europeans. In fact, the correlation between the C/C genotype (corresponding to lactose non-digesters) and positive breath test in unrelated family founders was significant. The genetic test compared to the breath test had a sensitivity of 95% and 91% and a specificity of 48% and 55% in adults and children, respectively.

2.6 Collaborations

We have a long standing collaboration with the Department of Life and Reproduction, Section of Genetics of the University of Verona, with the Section of Genetics of the Department of Clinical and Experimental Medicine of the University of Ferrara and with the Laboratory of Hematology & Clinical Chemistry of the University Hospital, Ferrara. Our Thalassemia Center is a part of the MIOT project, which is a network of 6 Magnetic Resonance Imaging apparatuses and 56 Italian Thalassemia Centers which share a common clinical and diagnostic database. In addition all the cooperative studies on Thalassemia and its complications are performed in collaboration with seven Italian Centers for the treatment of Thalassemia as listed in [2].

3 FUTURE PROJECTS AND TRANS2CARE NETWORKING

3.1 Mineral metabolism

In consideration of the work previously performed, as outlined above, we plan to clarify the bone mineral density, and the role of Vitamin D and the FGF23/Klotho axis in patients with thalassemia, in lactose intolerant children, in patients with gluten intolerance and in children treated with antiepileptic drugs. The components of the FGF23/Klotho axis have recently been recognized as important factors in the metabolism of calcium and phosphate. [13] Klotho functions as the obligate co-

receptor of FGF23. Mice lacking both FGF23 and Klotho develop identical phenotypes resembling premature aging syndromes, with hypogonadotropic hypogonadism, muscle and skin atrophy. The Klotho gene was originally identified as a putative age-suppressing gene in mice that extends life span when over-expressed. [14] In addition, Klotho functions as a humoral factor with pleiotropic activities including suppression of oxidative stress. [14] FGF23 regulates excretion of phosphates and synthesis of active Vitamin D in kidneys.[13]
Patients with thalassemia major exhibit many aging-like symptoms including hypogonadism, skin atrophy, muscle wasting osteopenia [15], reminiscent of the Klotho-deficient mice. Vitamin D metabolism and the FGF23/Klotho complex will therefore be studied in these patients and also in different populations of children affected by neurological and gastroenterological disorders.

Bone mineral density will be measured in patients by means of an ultrasonographic apparatus, acquired with Trans2Care funds. that spares the children studied the radiations inevitable with the more commonly used DEXA. [16].

3.2 Pseudoxanthoma elasticum in thalassemia

A subgroup of patients with thalassemia major or intermedia also suffer from Pseudoxanthoma elasticum, a condition in which widespread calcification of joints and arterial vessels develop without apparent cause unknown, [17] and in which the Klotho/FGF23 complex could be involved [18][19].
To clarify some of the aspects of this complication that (in the non-hereditary form) affects mainly patients with chronic hemolytic anemias and, in particular, patients with thalassemia major and intermedia, we plan to perform a PXE case-control study of the transcriptome using Next generation sequencing [20][21] of our patients with a and without PXE.

3.3 Tissue anti-transglutaminases in patients affected by seronegative gluten intolerance.

This part of the reaserch project will be conducted in collaboration with PP9. In fact, the pediatricians of the Burlo Garofalo Hospital in Trieste have already developed an expertise in this technique that we could use to plan a region-wide (Regione Emilia Romagna) study in seronegative symptomatic patients in whom gluten intolerance is suspected [22].

4 END SECTIONS

4.1 Acknowledgements

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5 CONCLUSION

During the last few years, the Pediatric Department of the University of Ferrara has conducted research in the field of the hemoglobinopathies and in the field of gastroenterology. We intend to pursue the same lines of research, enriching them with the addition of bone ultrasonography and the study of the newly described FGF23/Klotho system that promises to clarify some of the mechanisms at the basis of osteoporosis and extra-osseous calcifications in various diseases. Interweaving with other groups of the Trans2Care net will hopefully allow us to obtain more detailed information on our fields of interest that will provide improvements in prevention, early diagnosis and treatment of chronic diseases and their complications. The training of the researcher recruited by the project will contribute to increase the education and the internationalization of this educated young scientist.

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