Fibroblast growth factor 23 (FGF23) gene polymorphism in children with Kawasaki syndrome (KS) and susceptibility to cardiac abnormalities

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Abstract

Background: Fibroblast Growth Factor (FGF) 23 influences endothelial integrity and few reports have studied the association between FGF23 and Kawasaki syndrome (KS), a childhood vasculitis displaying a high risk of subsequent cardiac abnormalities (CaA).

Aim: To investigate the genetic variation in the FGF23 gene in a cohort of KS children and its association with serum FGF23 levels and eventual development of CaA, including both coronary artery dilatations and aneurysms.

Patients and methods: 84 Italian KS children were recruited; 24/84 (28.6%) developed CaA. Each patient underwent evaluation of serum FGF23 levels and FGF23 genotype: the frequency of the c.212-37insC (rs3832879) polymorphism in intron 1 was examined and compared with sex, age at disease onset, fever duration, laboratory data, and occurrence of CaA. Univariate statistical analysis of categorical parameters was performed by the Pearson’s Chi-square test or Fisher’s exact test as appropriate. Parametric variables were assessed by Student’s t-test for unpaired data. Independent predictors of disease were studied by a logistic regression model.

Results: 28/84 patients carried the FGF23 polymorphism (33.3%) and had higher serum FGF23 levels (p < 0.01). FGF23 polymorphism was significantly associated with CaA compared to wild type FGF23 children (respectively, p = 0.03 and p = 0.05). The comparison with demographical, clinical or laboratory data was not significant.

Conclusions: The prevalent segregation of the c.212-37insC polymorphism in children with CaA advocates a possible functional FGF23 role in the predisposition to higher serum levels of FGF23 and potential occurrence of any coronary artery abnormalities in KS.

Keywords: Kawasaki syndrome, Fibroblast growth factor 23, Cardiac abnormalities, Child

Kawasaki syndrome (KS), a systemic panvasculitis of unknown origin affecting all medium/small-sized vessels, typically occurring in early childhood, bears an outstanding risk of cardiac abnormalities (CaA), ranging from asymptomatic coronary artery ectasia to giant aneurysms [1]: these might occur in 15-25% of untreated cases during the subacute phase of KS, and an increasing body of evidence supports a widespread vascular dysfunction in KS with risk of atherogenesis during adulthood of these patients [2].

The discovery of fibroblast growth factor 23 (FGF23), a bone-derived hormone that regulates systemic phosphate homeostasis and vitamin D metabolism via FGF receptor 1 (FGFR1)/cofactor Klotho, which is included in a previously unrecognized hormonal bone-parathyroid-kidney axis [3], has uncovered new implications for skeletal fragility, renal disease-associated morbidity, and even cardiovascular pathology [4].
In the present study we have investigated the genetic variation in the FGF23 gene in a cohort of children with a confirmed diagnosis of KS and its association with serum FGF23 levels and development of CaA.

Patients and methods
Our study population included 84 consecutive children of Italian ancestry, 62 males and 22 females, with a mean age of 40.8 months, who fulfilled the American Heart Association criteria for the diagnosis of KS. Every patient was treated with intravenous immunoglobulin (IVIG, 2 g/kg over 10–12 hours within the 10th day since disease onset) and aspirin (50–80 mg/kg/day in 4 doses during the acute phase). All patients were immunoglobulin-responsive, and no repeated cycles of IVIG were required. No additional therapies were adopted. Fever duration (in days) was recorded for each patient. Endocrinological and chronic renal diseases, which can be associated with inappropriately high serum FGF23 levels or phosphate wasting and impaired bone mineralization, were excluded in all patients. Each patient underwent evaluation of serum FGF23 levels (expressed in pg/ml and measured by an ELISA assay, Immunotopics Inc. San Clemente, CA, USA; lower limit of detection: 1.0 pg/ml) at the time of inclusion in the study (i.e. when diagnosis of KS was formulated), in combination with the assessment of different laboratory data: erythroside-dimentation rate (ESR), C-reactive protein (CRP), haemoglobin (Hb), platelet count, fibrinogen, total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerids. All patients underwent 2D-echocardiogram at admission and routinely, if coronary artery involvement was absent. Twenty-four/84 KS patients (28.6%) developed CaA in terms of coronary artery complications (14 patients had coronary aneurysms and/or dilatations of whatever dimensions) and pericardial effusion (in 10 patients): all these children were more closely controlled by 2D-echocardiograms, performed always by the same pediatric cardiologist.

Fibroblast growth factor 23 gene evaluation
Both ethical approval from the Ethics Committee of the University of Florence and a formal informed consent by relatives or tutors of each child were obtained. Patients’ DNA was extracted from peripheral blood mononuclear cells. The three FGF23 exons, including the intron-exon boundary regions, were PCR-amplified and analyzed on ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Primer sequences were as follows: exon 1for GGGGTCTTTTGACATTTTCTTTC; exon 1rev GGTT GGATTAGCCCCTCAG; exon 2for ATCAATCCAGG GAGGTTTCA; exon 2rev GGAAACAGGTCAACCCAGG GTA; exon 3for AGGAGGAGCTGGGGAGTG and exon 3rev GACCTGGTCCCTTGGGAAGA. PCR was performed with 1.5 mM magnesium chloride, 0.2 mM deoxynucleotide triphosphates, 0.2 µM of each primer, 1 U of Taq polymerase and 100 ng of genomic DNA as template. The obtained sequences were compared with the wild type reference sequence of the gene, published on Genbank Database (NT-009759). FGF23 gene analysis revealed the polymorphism c.212-37insC in intron 1, characterized by a C insertion in the intronic region between –36 and –37 nucleotide (rs3832879: NM_020638.2:c.212-37_212-36insC), in a subset of KS patients. Subjects without this FGF23 polymorphism were indicated as wild type.

Statistical analysis
Univariate statistical analysis of categorical parameters was performed by the Pearson’s Chi-square test or Fisher’s exact test as appropriate. Parametric variables were assessed by Student’s t-test for unpaired data. The comparison of age of onset between the two groups was performed by Mann–Whitney U test. Variables significantly associated with CaA at the univariate analysis were entered into a logistic regression model to identify independent predictors of disease. Values were expressed as total count [% ratio] for categorical variables, means ± SD for parametric variables and value [95% Confidence Interval] for Odds ratio. Age of onset was expressed as median [quartiles]. A p value of 0.05 or less was considered as significant.

Results
Twenty-eight patients with KS carried the FGF23 c.212-37insC polymorphism (33.3%). We found that male sex was significantly associated with the polymorphism (91.7% vs 61.7%, p = 0.03). Patients presenting the FGF23 polymorphism had also significantly higher serum FGF23 levels (41.4 pg/ml ± 53.9 vs 10.7 pg/ml ± 13.5, p < 0.01). In addition, the FGF23 polymorphism was significantly associated with CaA: indeed, patients presenting with coronary dilatations or aneurysms were significantly more represented in the subgroup with FGF23 polymorphism, compared to wild type FGF23 children (29.2% vs 11.7%, p = 0.05). The number of patients with only coronary aneurysms (3 patients) was too small to obtain statistical significance in a separate subgroup analysis. Neither pericardial effusion, nor the overall signs of cardiac involvement (in the whole) were significantly associated with the FGF23 polymorphism. No demographic, clinical or laboratory data were found to be associated with the FGF23 polymorphism.

At the multivariate analysis, FGF23 polymorphism was independently associated with both CaA and high serum FGF23 levels (respectively Odds ratios: 4.3 [1.1–16.2], p = 0.03 and 1.05 [1.0–1.1], p = 0.01). Male sex, that showed a strong univariate association with the FGF23 polymorphism, did not reach a statistical significance at
the multivariate analysis: however, since the FGF23 polymorphism was mostly observed in males (22 out of 24, 91.7%), we probably have not sufficient data to determine its real impact in the male sex (see Table 1).

Discussion

Endothelium dysfunction is an established trigger for cardiovascular accidents in the general population and is a major pathogenetic mechanism involved in KS [5]. FGF23, which is part of hormonal bone-parathyroid-kidney axis, modulated by parathyroid hormone, 1,25 (OH)2-vitamin D, diet and serum phosphorus levels, exerts its bioactivity on selected target-tissues interacting with its receptor FGFr, largely diffuse in bone tissue and different endothelia, in the presence of Klotho co-factor [6]. Elevated serum levels of FGF23 have been demonstrated in children with rickets and diminished bone mineral density, but also a direct FGF23 effect has been hypothesized on the heart [7,8]. In a previous study we have observed that intact serum FGF23 levels in children with KS were significantly higher when compared with healthy controls, in particular those displaying CaA, revealing the potential role of FGF23 as a marker suggestive of cardiac complications [9]. Unfortunately there are no other published studies on the association between vasculitides in childhood and FGF23, and clinical trials are needed to determine whether FGF23 is a modifiable risk factor.

In this study we have investigated the genetic variation in the FGF23 gene in a cohort of 84 children with KS and its correlation with serum FGF23 levels and development of CaA: we have found a significant correlation between the FGF23 rs3832879 variant (which can be found in 15.6% of the population; http://www.ncbi.nlm.nih.gov/snp/?term=rs3832879) and both serum FGF23 levels and CaA, including both coronary artery dilations and aneurysms. In particular, patients with the FGF23 polymorphism had higher serum FGF23 levels

Table 1 Univariate statistical analysis with demographic/laboratory variables and cardiac abnormalities in comparison with the FGF23 gene polymorphism in the cohort of 84 children with Kawasaki syndrome recruited for this study

| Variable                        | FGF23 wild gene | FGF23 polymorphism | p value |
|---------------------------------|-----------------|--------------------|---------|
| Sex (M/F)                       | 40/20           | 22/2               | 0.03    |
| Age of onset (in months)        | 24.5 [13.2-49.5]| 36 [16.7-58.0]    | 0.39    |
| Fever duration (in days)        | 8.7 ± 4.0       | 9.3 ± 3.8          | 0.75    |
| **Laboratory data**             |                 |                    |         |
| Serum FGF23                     | 10.7 ± 13.5     | 41.4 ± 53.9        | 0.01    |
| ESR at onset (mm/1 h)           | 83.3 ± 22.7     | 83.5 ± 19.4        | 0.44    |
| CRP at onset (mg/L)             | 14.0 ± 15.8     | 17.3 ± 16.9        | 0.52    |
| Hb at onset (g/L)               | 10.6 ± 1.1      | 10.2 ± 0.9         | 0.48    |
| Platelet count at onset (x10^9/L)| 450.8 ± 142.1   | 433.0 ± 124.1      | 0.86    |
| Fibrinogen at onset (mg/dL)     | 672.3 ± 216.6   | 732.6 ± 162.3      | 0.27    |
| Total cholesterol (mg/dl)       | 162.2 ± 34.0    | 171.8 ± 223        | 0.13    |
| HDL cholesterol (mg/dl)         | 39.5 ± 14.6     | 39.6 ± 12.5        | 0.59    |
| LDL cholesterol (mg/dl)         | 103.7 ± 39.7    | 105.6 ± 29.6       | 0.44    |
| Triglycerides (mg/dl)           | 86.5 ± 58.1     | 89.7 ± 76.4        | 0.65    |
| **Cardiac abnormalities**       |                 |                    |         |
| Coronary dilatations            | 6/54            | 7/17               | 0.03    |
| (present/absent)                | (10.0%/90.0%)   | (29.2%/70.8%)      |         |
| Coronary aneurysms               | 2/58            | 1/23               | 0.64    |
| (present/absent)                | (3.3%/96.7%)    | (4.2%/95.8%)       |         |
| Coronary aneurysms or dilatations| 7/53            | 7/17               | 0.05    |
| (present/absent)                | (11.7%/88.3%)   | (29.2%/70.8%)      |         |
| Pericardial effusion            | 7/53            | 4/20               | 0.54    |
| (present/absent)                | (11.7%/88.3%)   | (16.7%/83.3%)      |         |
| Any cardiac involvement         | 14/46           | 10/14              | 0.09    |
| (present/absent)                | (23.3%/76.7%)   | (41.7%/58.3%)      |         |

All parametric data are expressed as mean ± SD. Age of onset is expressed as median [quartiles]. Categorical variables are expressed as raw numbers (%).
(p < 0.01) and developed more frequently coronary artery dilatations or aneurysms (p = 0.05). Finally, the multivariate analysis showed that both CaA and serum FGF23 were independently associated with the FGF23 polymorphism (respectively p = 0.03 and p = 0.01).

In summary, coronary artery dilatations were demonstrated in 29.2% of children bearing the FGF23 polymorphism and only in 10% of wild type FGF23 patients: this striking difference might support the functional role that this polymorphism could display on the susceptibility to CaA, although the details of the interaction of FGF23 gene with other genetic systems remain unclear at present.

From these preliminary results, which need to be replicated on larger samples of patients, the segregation of the FGF23 polymorphism in children with KS who developed CaA suggests its potential contribution to the development of cardiovascular complications. Whether this genotype may act in synergy with other genes or specific environmental factors remains to be elucidated in further studies.

Abbreviations
FGF23: Fibroblast Growth Factor 23; KS: Kawasaki syndrome; CaA: Cardiac abnormalities; IVIG: Intravenous immunoglobulins; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; Hb: Haemoglobin.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
FF (submitting author), LM, MMC, and MLB primarily created the protocol of the study; FF and DR drafted the manuscript and revised it based on all co-authors’ suggestions; GT and MC made the statistical analysis of the study; FF and GL performed the genetical assays in patients recruited in the study. FF and DR equally contributed to the overall production of this study and manuscript. All authors read and approved the final manuscript.

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