FRI0011

A TRANSCRIPTIONAL REGULATOR CONTROLLING SEVERITY OF EXPERIMENTAL ARTHRITIS

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Background: Susceptibility to Rheumatoid Arthritis (RA) is dependent on complex interactions among genetic and environmental factors. Protein candidates and their role in pathways leading to chronic inflammation of the joints, in addition to their potential as drug targets, can be revealed with the help of experimental models for disease (1). From the results of functional genetic studies, we have recently shown that the T-box gene, *TBX3*, is a candidate gene in Collagen Induced Arthritis (CIA), an experimental model for RA (2). *TBX3* encodes a transcriptional regulator involved in differentiation of several organs, including bone, during embryonic development. It has, in addition, been demonstrated important in oncogenesis (3). Our studies suggest that *TBX3* has a role in B-cell activation and is important for the severity of disease in the CIA model (2).

Objectives: The objective of this project is to understand the role for the transcriptional regulator *TBX3* in development of RA.

Methods: Bioinformatics based comparative studies of mouse and human alleles in the regulatory region of *TBX3*. CRISPR/Cas9-introduced deletions and base modifications in human B-cell lines. Activation of genetically modified B-cells in vitro, followed by analyses of proliferative response and antibody production.

Results: Studies of CIA development in mice with single nucleotide polymorphisms (SNPs) in the regulatory region of *Tbx3* revealed a significant difference in severity of arthritis. In line with this, the anti-collagen type II antibody titers were shown substantially higher in mice with more severe arthritis, even before onset of disease. In addition, preliminary data shows that the proliferative response to Type II collagen upon re-challenge of lymph node cells in vitro is higher in these mice, suggesting a more active response to the disease-inducing antigen. Because the *TBX3* gene is conserved between mouse and human, we are investigating whether similar genetic variations are found in the regulatory region of the human *TBX3* gene and whether the putative genetic variation would lead to a distinct B-cell phenotype upon activation in vitro.

Conclusion: We suggest that the oncoprotein *TBX3* is a novel candidate contributing to disease severity in experimental arthritis. Investigations of genetic variation in the *TBX3* gene and its role in the activation of human B-cells will reveal whether this protein is a candidate for influencing also development of RA.

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FRI0012

THE CLINICAL SPECTRUM AND PEDIGREE ANALYSIS OF TRAPS IN GREECE, INCLUDING A NOVEL MUTATION-RESULTS FORM A NATIONAL REFERRAL CENTRE

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Background: Tumor necrosis factor receptor-associated periodic syndrome (TRAPS) stems from autosomal dominantly inherited mutations in the *TNFRSF1A* (accession number: NM_001065) gene, encoding for the receptor of tumor necrosis factor α (TNFR1).

Objectives: The aim of the study was to report a novel *TNFRSF1A* mutation and to describe the clinical phenotypes in families carrying different *TNFRSF1A* mutations.

Methods: Four Greek patients with TRAPS-like clinical features were evaluated for *TNFRSF1A* gene mutations. Direct sequencing of exons 2, 3 and 4 of the gene was performed. Following positive testing of the index cases, samples from other family members were collected and screened.

Results: A total of eighteen members deriving from four unrelated Greek families were investigated. In the first family, a novel (heterozygous) mutation in cysteine residue in exon 3 of the *TNFRSF1A* gene C73Y (c.305G>A) was identified in three members. Interestingly, in the same family a patient carrying the low penetrance *TNFRSF1A* R92Q mutation, as well as a patient with concomitant R92Q and C73Y mutations were identified. In the second family, the *TNFRSF1A* C73W (c.306C>G) heterozygous mutation was identified in seven members. In the third family, the *TNFRSF1A* T50M mutation was detected in two members while in the fourth family six members carried the *TNFRSF1A* R92Q mutation in heterozygous state.

Clinical manifestations amongst members of the affected families were diverse with the most serious being present in patients carrying the *TNFRSF1A* C73Y, C73W and T50M mutations. Cardinal features included disease onset in childhood (66.7% for C73Y, 85.7% for C73W and 100% for T50M), arthritis (67% for C73Y, 100% for C73W and 100% for T50M), persistent pyrexia (67% for C73Y, 100% for C73W and 100% for T50M), abdominal pain (66.7% for C73Y, 100% for C73W and 100% for T50M), recurrent adhesive ileus (33.3% for C73Y, 14.3% for C73W and 100% for T50M) anterior uveitis (33.3% for C73Y), and diffuse maculopapular rash (14.3% for C73W and 50% for T50M). The clinical presentation was more severe in the patient with concomitant R92Q and C73Y, suggesting an additive effect. On the contrary, (as expected) the disease spectrum associated with R92Q mutation found in six members encompassed a mild phenotype, extending from asymptomatic state (four members) to adult-onset disease associated with intermittent low-grade fever, arthritis and elevated inflammation markers (two members).

Conclusion: This was the first pedigree analysis of TRAPS in Greece, depicting four families with unique mutations, along with analysis of the clinical manifestation. The site of mutation might explain the diversity of clinical phenotypes in TRAPS patients that extends from mild to severe disease. In our patients, it appeared that mutations in the cysteine residues as well as in T50M were associated with more severe clinical manifestations. Among these was a novel mutation described for the first time in the literature.

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FRI0013

ABSTRACT WITHDRAWN

FRI0014

GENETIC VARIABILITY IN MOLECULES REGULATING BONE REMODELING. DO THEY INFLUENCE SEVERITY OF DISEASE AND BONE MASS IN PATIENTS WITH EARLY-ONSET ARTHRITIS?

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Background: In the last few years, an association has been described between lower bone mass at the onset of disease and higher severity of rheumatoid arthritis (RA).

Objectives: To identify single nucleotide polymorphisms (SNPs) in genes related to bone remodeling associated with severity of disease and bone mineral density (BMD) in patients with early-onset arthritis.

Methods: We included 268 PEARL (Princess Early Arthritis Register Longitudinal) patients genotyped with Illumina Inc. Immunochip. This array includes 556 SNPs with different density levels in semaphorins 4b, 4d and 4f, DKK1, 2 and 3, sclerostin, osteoprotegerin (OPG), RANK and

RANKL. All SNPs with genotyping rate <98%, with frequency of the minor allele <1% and those that were not in Hardy-Weinberg equilibrium, were excluded. In addition, among the SNPs in linkage disequilibrium, a representative one was chosen, so that only 159 were finally studied. The intensity of treatment, the activity of the disease measured by the DAS28 and HUPI indices, as well as having achieved at least low activity after 2 years of follow-up were chosen as surrogate severity variables. The association of each of the SNPs with these variables was analyzed by linear regression, and ordered logistic regression according to the variable, and adjusted for potentially confusing variables, such as age, sex and presence of anti-cyclic citrullinated peptide antibodies. We selected those SNPs that were consistently associated with ≥ 3 surrogate severity variables, and then, the relationship of the selected SNPs with BMD of the forearm, lumbar spine and hip (Hologic-4500 QDR) was analyzed by means of linear regression adjusted for age and sex.

Results: As shown in the attached table, 11 SNPs were associated with the severity of the disease; 7 of them were also associated with BMD, although only 3 confirmed the previously described relationship between severity of disease and BMD.

SNP	GENE	MOLECULE	DISEASE ACTIVITY	BMD
rs4355801	LOC441377/ TNFRSF11B	OPG	Decrease	No relationship
rs3134058	TNFRSF11B	OPG	Increase	No relationship
rs10505346	TNFRSF11B	OPG	Increase	No relationship
rs6469804	TNFRSF11B/ COLLECT10	OPG	Decrease	No relationship
rs1805034	TNFRSF11A	RANK	Decrease	Decrease
rs599323	TNFRSF11A/ZCCHC2	RANK	Increase	Increase
rs386283	AKAP11/TNFSF11	RANK-L	Increase	Increase
rs1324004	AKAP11/TNFSF11	RANK-L	Decrease	Increase
rs111900837	AKAP11/TNFSF11	RANK-L	Increase	Decrease
rs7142875	AKAP11/TNFSF11	RANK-L	Increase	Increase
rs7557277	AKAP11/TNFSF11	RANK-L	Increase	Decrease

Conclusion: This preliminary study highlights the relationship between gene variants of molecules involved in bone remodeling and the severity of early-onset arthritis. However, more studies are needed to confirm this relationship and validate it in other populations. This would confirm the involvement of these gene variants in disease activity and bone mass variation.

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FRI0015

EXON 5 DUPLICATION IN ALPL GENE AS THE GENETIC CAUSE OF HYPOPHOSPHATASIA IN A 3 YEAR OLD GIRL

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Background: Hypophosphatasia (HPP) (OMIM 146300, 241510, 241500) is an inborn error of metabolism caused by pathogenic variants in the gene *ALPL* that codifies for the tissue non-specific alkaline phosphatase (¹). The disease presents with different degrees of severity, from perinatal forms to mild adult phenotypes, including odontohypophosphatasia where only dental complications are described. Until now, more than 380 variants are described to cause HPP, and only 2.9% are large deletions or duplications (http://www.sesep.uvsg.fr/03_hypo_mutations.php).

Objectives: Identify the genetic cause of the disease in the girl with an infantile HPP.

Methods: The patient is a female child born by cesarean at week 37 of gestation. The child required reanimation. Weight, length and cranial perimeter were below percentile 10th (1735g, 42 cm, 31.5 cm respectively). She presented and obstetric femur fracture and normal to low alkaline phosphatase (AF) activity (158 U/L (reference 142-335 U/L)). After fracture, a second determination of AF (127U/L) and pyridoxal-5'-phosphate (PLP) concentration (240.41 mgc/L, reference 6.4-23) were measured. Skeletal interrogation revealed low bone mineralization, tongues of radiofluency in long bones and narrow ribs. A clinical diagnose of hypophosphatasia was done. At the three year-old revision she still presents weight and length below percentile 15th, a mild cardiac problem but had no fractures since birth. She presents lung malformation, and cannot eat solid food. She still has low AF activity (154 U/L) and high PLP (69.13 µg/L; references 150-350 U/L and 3.6-18 µg/L respectively). She is on school but has language delay. Genetic analysis: DNA was extracted from peripheral blood of patient and her mother. From patient's sample we performed Sanger sequencing of exons 1 to 12, CGH analysis using a customized whole genome CGH 4x44k Agilent array enriched in probes for *ALPL* gene (130 probes) and qPCR of exons 2 to 12.

Results: The study of copy number variants of *ALPL* gene by qPCR revealed a duplication in exon 5 in the patient and her mother.

Conclusion: Although gross deletions and duplications are a rare phenomenon in HPP, here we present a case of a 3 year old child and her mother that present a single exon duplication as a probable cause of the disease. Actually, the mother is under clinical evaluation. Part of the active site is codified in exon 5, suggesting that a duplication might alter the correct structure and/or function of the protein. So, including the screening of copy number variants during the genetic screening is important to reach the correct diagnosis of HPP.

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