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Case Report / Приказ случаја

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TNFRSF1A gene variant identified in a boy with recurrent episodes of fever

Варијанта гена *TNFRSF1A* код дечака са рекурентном фебрилношћу

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SUMMARY

Introduction Fever of unknown origin is an important diagnostic challenge. Although rare, periodic fever syndromes may often present with a chronic or recurrent febrile condition with a variable temporal pattern of ocurrence. Although clinical characteristics often indicate the syndrome in question, there are many atypical forms, and the genotype-phenotype relationship is highly complex, warranting in many cases the designation of a "syndrome spectrum" rather than a syndrome *per se*.

The aim of this was to hereby present a boy with recurrent fever of unknown origin.

Case outline A boy with recurrent fever of unknown origin who was by clinically guided partial exome sequencing found to have a heterozygous variant 434A>G in the *TNFRSF1A* gene, otherwise connected with TRAPS. The patient responded well to short courses of glucocorticoids and is no longer subjected to unnecessary antibiotic treatment he had frequently received in the past.

Conclusion Periodic fever syndromes should be kept in mind as a differential diagnostic possibility in children with fever of unknown origin.

Keywords: fever; autoinflammatory disorders; TRAPS; genotype-phenotype correlation

САЖЕТАК

Увод Нејасно фебрилно стање је значајан дијагностички изазов. Мада су ретки, синдроми повремене грознице се често могу испољити у виду фебрилног стања које се понавља у различитим интервалима и траје краће или дуже време, или је пак хронично. Премда клиничка слика неретко указује на синдром о коме се ради, однос генотипа и фенотипа је веома сложен, што често оправдава сврставање у „спектар синдрома" уместо у посебан синдром.

Циљ овог рада је био да прикажемо дечака са понављаним налетима грознице непознатог узрока.

Приказ болесника Дечак са понављаним налетима грознице непознатог узрока код кога је клинички усмерено делимично секвенцирање егзома показало хетерозиготну варијанту 434A>G у гену *TNFRSF1A*. Овај ген је иначе повезан са синдромом TRAPS. Пацијент је показао добар терапијски одговор на краткотрајно давање глукокортикоида и више се не лечи дуготрајним давањем антибиотика, што му је често ординирано у прошлости.

Закључак У диференцијалној дијагнози код деце са грозницом непознатог узрока ваља имати на уму и синдроме повратне грознице.

Кључне речи: грозница; аутоинфламаторне болести; TRAPS; однос генотип-фенотип

INTRODUCTION

Prolonged or recurrent fever of unknown origin constitutes an important diagnostic problem that may be connected to a multitude of potential etiological factors and nosological entities [1, 2]. Although exceedingly rare, autoinflammatory disorders often present with a febrile condition of unknown origin. Depending on the syndrome in question, febrile spells may occur in relatively regular intervals, or there may be no discernible time pattern in their ocurrence [3, 4]. Of all periodic fever syndromes, PFAPA syndrome (Periodic Fever, Aphtous stomatitis, Pharyngitis and Adenitis) exhibits by far the greatest incidence. However, this syndrome is not readily explained by a defined genetic aberration(s) and is thought to be an etiologically (and to some extent also pathogenetically) heterogenous category, diagnosed *per exclusionem* by the presence of fever and inflammation in a person without signs of infection and with a prompt resolution of symptoms upon glucocorticoid treatment [5]. Most common periodic fever syndromes of autoinflammatory nature with fully characterized genetic causes include familial mediterranean fever (FMF), cryopyrin-associated periodic syndromes (CAPS), mevalonate kinase deficiency (MKD)/hyperimmunoglobulinemia D and periodic fever syndrome (HIDS), and tumor necrosis factor receptor-associated periodic fever syndrome (TRAPS) [6]. Symptoms and signs other than fever, such as joint pains, skin rash, serositis or abdominal aches may be very helpful in the establishment of diagnosis [7].

Table 1. Autoinflammatory Disorder Activity Index (AIDAI).

a. Fever ≥ 38 °C
b. Overall symptoms
c. Abdominal pain
d. Nausea/vomiting
e. Diarrhea
f. Headaches
g. Chest pain
h. Painful nodes
i. Arhralgia or myalgia
j. Swelling of the joints
k. Eye manifestations
l. Skin rash
m. Pain relief taken

FMD: a+c+g+i+j+l;
MKD: a+c+d+e+h+i:
TRAPS: a+b+c+i+k+l;
CAPS: a+f+i+k+l.
All variables are scored 0-3, except fever (0 or 1).
Monthly AIDAI is a sum of 31 daily values.

Treatment partly depends on the syndrome in question and in many cases may be successfully guided by clinical tools such as the Auto-Inflammatory Diseases Activity Index (AIDAI; Table 1) [8]. Early and appropriate treatment can greatly reduce the risk of complications, including the most serious, amyloidosis [9]. It is therefore of utmost importance to systematically evaluate children with fever of unknown origin for possible autoinflammatory disorders. If performed thoroughly, this very often results in a precise diagnosis [10]. We hereby present the case of a boy with recurrent episodes of fever that were eventually plausibly explained by the result of genetic testing.

CASE REPORT

Repeated instances of febrile illness in an otherwise healthy boy began at the age of 6 years. They were separated by an interval of several months. Bodily temperature usually reached 40 °C, while C-reactive protein (CRP) was in the range 40-100 mg/L, and the erythrocyte sedimentation rate (ESR) typically about 30 mm/h. A mild splenomegaly has also been noted. Febrile episodes lasted from a few days (usually about seven) to several weeks. Antimicrobial treatment had no effect on the time to resolution.

Before the onset of disease, patient's personal history was unremarkable. He received all obligatory vaccines according to the official vaccination schedule in the Republic of Serbia. He did not suffer from any allergies. His mother and brother were allergic to pollen, and both parents, as well as the paternal grandparents, suffered from cardiovascular disorders at a relatively young age (most of them in the fifth decade of life). No other diseases or problems were reported.

On three occasions (at the age of 8, 11, and 12 years, respectively) the boy was hospitalized an another pediatric tertiary center for a detailed diagnostic investigation. Repeated ultrasound (US) and magnetic resonance imaging (MRI) examinations confirmed a persistent, although mild hepatosplenomegaly and slightly enlarged retroperitoneal and mesenteric lymph nodes. However, peripheral lymphadenopathy was absent at all times. During some of the febrile episodes, the boy also complained of joint pains, particularly in the left temporomandibular joint. These were never accompanied by any other signs of arthritis. On one occasion he also felt acute pain in the heel, indicating a possible bout of enthesitis. He never had a skin rash, but occasionally suffered from eye irritation and redness. When febrile, the patient was usually prescribed prolonged courses of broad-

spectrum antibiotics, lasting up to 21 days. Between the febrile episodes he was quite well, and participated in sporting activities at school.

Laboratory examination yielded a mild chronic anemia (hemoglobin 12-13 g/L); during febrile intervals, he often also had a borderline thrombocytopenia (typically 90-100 x 10^9/L), but never any leukocytosis. Total protein, albumin, glucose, urea, creatinin, electrolytes, transaminases, bilirubin, alkaline phosphatase, γ-glutamyl transferase, creatin kinase and α-amylase were at all times within the reference range. The results of urine analysis were also normal. Plasma immunoglobulin levels were within the age-specific reference range, including IgD. Extensive autoantibody testing (ANA, ANCA, ASCA, anti-LKM, ASMA, dsDNA, anti-tTG, anti-endomysial, anti-Tg, anti-TPO) showed completely negative results. C3 and C4 levels were normal. Bone marrow biopsy (also at the age of 8) gave a normal result, as did karyotype analysis (46, XY). During the detailed endocrinological examinations, a mild elevation of total cholesterol (7,4 mmol/L) and LDL (2,98 mmol/L) was detected in the plasma, as well as that of cortisol. Thyroid hormones were at all times within the normal range, and so was TSH. Plasma ceruloplasmin, ACE, and fecal calprotectin were also at normal values, as were CEA, NSE and AFP. The ECG and the cardiac US examination revealed no abnormalities. The same was true for the ear, nose and throat specialist examination. Virus serology testing detected anti-EBV IgG antibodies, while anti-HCV antibodies and anti-CMV antibodies (both IgG and IgM) were absent. HBsAg was also found to be absent. Purified protein derivative (PPD) testing for tuberculosis yielded a negative result. Given the absence of pharyngitis, cervical lymphadenitis, aphtous stomatitis, and the appropriate temporal pattern of fever, the patient never satisfied the diagnostic criteria for PFAPA syndrome, although the possibility of an atypical form has repeatedly been considered in differential diagnosis.

At the age of 14 he came to our attention and two new febrile episodes separated by three months were successfully and promptly terminated by a short (~ 4 days) course of glucocorticoids. This time a bilateral acute uveitis also appeared, and was subsequently shown to be of granulomatous nature by slit lamp examination. It responded well to topical glucocorticoid treatment. On US examination, the spleen reached a maximal craniocaudal diameter of 145 mm. At this time a monogenic autoinflammatory disorder was first suspected. Considering the presence of uveitis, investigations were initially directed toward a highly atypical form of systemic juvenile arthritis. However, HLA typing excluded the presence of the B27 allele, while MRI showed no lesions of sacroiliac joints that would be indicative of spondyloarthropaties. Serum amyloid A concentration was measured within physiological limits. Given the great number of potential genetic alterations that are known to fit the clinical presentation and disease course, clinically guided partial exome sequencing was undertaken, with an emphasis on genes with a known function connected with autoinflammatory disorders. Partial exome analysis, performed at University Clinical Center Ljubljana, Slovenia, revealed the existence of heterozygous variant in the *TNFRSF1A* gene (*TNFRSF1A*: c. 434A>G).

At the time of writing, the patient is feeling well and has no symptoms. In the meantime he experienced only one episode in the period of 14 months. His spleen completely receded to physiological bounds and is now 127 mm in AP diameter. His most recent monthly AIDAI (Table 1) was 20, as compared to 108 at the time of peak disease activity. The patient is also instructed to undego yearly US examinations and routine blood analyses of inflammatory parameters.

DISCUSSION

Clinically, our patient exhibited clear signs of a long-standing inflammatory condition with elements of some autoinflammatory disorders (episodes of fever, joint pains, splenomegaly, possible enthesitis of the Achilles' tendon, uveitis, prompt response to glucocorticoids). However, diagnostic criteria for any specific disorder were not satisfied, suggesting either an extremely rare nosological entity or some rather untypical clinical variant of a more common one. Bearing in mind that it is usually. if not universally, reasonable to assume that the latter is vastly more likely that the former, we decided it is worthwhile to perform clinically guided partial exome sequencing in order to identify potential gene variants that could explain the observed symptoms and signs.

Mutations in *TNFRSF1A* cause TNF receptor-associated autoinflammatory syndrome (TRAPS) [11]. TRAPS was formerly called "familial Hibernian fever" because it had initially been described in a family of Scottish ancestry (Hibernia being the Roman name for Scotland) [12]. *TNFRSF1A* encodes a member of the TNF receptor superfamily and is therefore extensively involved in inflammatory processes associated with both innate and adaptive immune mechanisms and processes. It is mainly expressed on mononuclear phagocytes, but may also be found on a number of other cell types, such as lymphocytes, natural killer (NK) cells, granulocytes, astrocytes and keratinocytes [13]. Numerous different variants in *TNFRSF1A* have been described, with a highly complex genotype-phenotype relationship [14]. Prognosis is variable and primarily dependent on the existence of complications of chronic inflammation, such as amyloidosis. Variant 434A>G is recorded in the ClinVar database (No. 97703) [15] and Infevers registry [16], and designated as a genetic variant of unknown significance. However, the same variant has been reported in a patient listed in the EUROFEVER registry with clinically apparent TRAPS [17].

Although there is no possibility of final proof that the detected gene variant indeed plays a causal role in our patient's ailment, it is certainly plausible that it has at least some effect, based on obvious pathophysiological mechanisms (*i. e.*, uncontrolled inflammation) and known functions of the *TNFRSF1A* gene (including inflammatory signaling). Considering the relatively inocuous disease course so far and the absence of any signs of permanent organ damage or amyloidosis, the outlook for our young patient appears to be favorable. The example we describe here could be used as a good illustration of the concept of "genomic landscape" of congenital autoinflammatory (as well as other) syndroms; the complexity of this landscape very often does not allow a clear demarcation line to be drawn between a pathological and a physiological gene variant, particularly when seen in the light of

the less-than-predictable relationship between the nature of genetic alteration and its clinical consequences, if any. Low-penetrance *TNFRSF1A* variants are well known and appear to cause a mild or moderate autoinflammatory condition in some, though not all, affected persons [18]. Furthermore, the low-penetrance variants appear to produce their effects through a different pathophysiological mechanism compared with clearly pathogenic gene alterations, and are, at least in part, connected to the functional status of regulatory T cells [19]. An analysis of a series of patients who carry a well characterized low-penetrance variant has shown that severity of symptoms and risk of complications are highly variable and at least partially correlated with the age of onset [20]. In the light of all this, it appears quite justified to speak of a "TRAPS spectrum" as a diagnostic category (as opposed to the diagnosis of TRAPS *per se*). The rationale for using the latter designation appears rather strengthened by the fact that our patient constantly exhibited some, but never all, features of PFAPA syndrome, begging the question how many patients classified within this highly heterogenous diagnostic category are (or were) actually affected by *TNFRSF1A* variants, among other defects in genes connected to inflammation. This and a myriad of analogous possibilities in other autoinflammatory disorders, such as, for instance, FMF, CAPS and MKD (to name the most frequent ones in our population, aside from TRAPS) warrants particular attention when the physician is faced with a patient clinically exhibiting some, but not all features of a known autoinflammatory syndrome. In such instances, clinically guided partial exome sequencing, if available, generally tends to become the diagnostic method of choice. On the other hand, it is an exceedingly costly and somewhat time-consuming procedure, and this adds to the importance that all physicians, and especially pediatricians, be satisfactorily acquainted with the full range of clinical situations where it is rational to order such an analysis. This appears to be of the essence, since the usefulness of extensive genetic testing without proper clinical guidance is very doubtful, as highlighted, for instance, by the recently published experience in autoinflammatory disorders from a center in Trieste [21]. A deeper knowledge of possible genetic alterations and their complex consequences should ensure the necessary amount of critical thinking in determining whether testing is indicated or warranted, and this is, indeed, more than appropriate for the practice of medicine in the genomic age.

The distinction between TRAPS and the proposed designation of "TRAPS spectrum" can also be viewed as highly meaningful from the treatment standpoint. While TRAPS patients are usually best treated with IL-1 antagonists such as anakinra [22, 23] or canakinumab [24], most patients with low-penetrance *TNFRSF1A* alterations either require no treatment or sufficient disease control can be achieved by occasional short courses of glucocorticoids, administered as needed [18].

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