Case Report

Blau Syndrome Mutation of *CARD15*/*NOD2* in Sporadic Early Onset Granulomatous Arthritis

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ABSTRACT. Patients with sporadic early-onset granulomatous arthritis are clinically identical to Blau syndrome, but without the family history. Blau syndrome is an autosomal dominant inherited disease and is known to be caused by mutations in the *CARD15* gene (also called *NOD2*). We investigated the hypothesis that an individual with sporadic early onset granulomatous arthritis may have a Blau syndrome mutation in *CARD15*/*NOD2*. Our patient's genomic DNA isolated from a buccal swab sample was subjected to amplification to include the region of exon 4 from the *CARD15*/*NOD2* gene that contains known mutations that cause Blau syndrome. This region was screened for mutations by direct DNA sequencing in both directions. One of the mutations in *CARD15*/*NOD2* attributed to Blau syndrome was found in the DNA sample. The nucleotide change encodes an amino acid substitution from arginine to tryptophan at position 334 of the protein. This mutation has been found in some Blau syndrome pedigrees reported in the literature. These data suggest that sporadic granulomatous arthritis may in fact be the sporadic form of Blau syndrome, but arising from a spontaneous neomutation. This would explain the profound clinical identity and the lack of disease history in the parents. (J Rheumatol 2005;32:373–5)

Key Indexing Terms:
SARCOIDOSIS BLAU SYNDROME CARD 15 ARTHRITIS UVEITIS

Early onset granulomatous ("sarcoid") arthritis is a multisystemic disease characterized by symmetrical polyarticular exuberant synovitis, a characteristic scaly rash, and uveitis. Onset before age 5 years is typical and antinuclear antibodies (ANA) are conspicuously absent. Diagnosis is confirmed by the presence of noncaseating granulomas interspersed with chronic inflammatory cells infiltrating dermis and synovium. Its mechanism remains unknown. Most investigators agree that early onset granulomatous arthritis is clinically distinct from adult sarcoidosis, in that the latter commonly includes lung involvement and hilar adenopathy, while the former usually presents with arthritis and scaly rash[1,2].

An autosomal-dominant familial disease involving granulomatous synovitis and uveitis was described by Jabs, *et al*[3], and Blau[4] in 2 separate studies the same year, with the latter reporting involvement of the skin as well. The resemblance between early-onset sarcoid arthritis and Blau syndrome led to foundational work proposing that the 2 diseases represented the same condition[5]. We as well postulated a common disease mechanism[6].

Two amino acid substitutions at position 334 (R334W and R334Q) of CARD15/NOD2 and one at position 469 (L469F) were initially described by Miceli-Richard, *et al* and confirmed by others in 50% of families studied with Blau syndrome[7,8]. Two additional individuals with nonfamilial sarcoidosis, involving kidney granulomas in one and lymphocytic alveolitis in the other, were studied by Miceli-Richard *et al*, but a disease-causing mutation was not found[7]. This is consistent with the conclusion by Rybicki *et al* that the Blau syndrome locus where CARD15/NOD2 resides is not involved in genetic predisposition to sarcoidosis[9]. Recently, Schurmann, *et al* analyzed a cohort of

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patients with adult-onset sarcoidosis and found no evidence for an association between *CARD15*/*NOD2* mutations and sarcoidosis, including the known Blau syndrome mutations[10]. Finally, disease-associated mutations in *CARD15*/*NOD2* were not found in a cohort of patients with sarcoidosis-associated uveitis[11].

We describe the first case of a sporadic form of early onset granulomatous arthritis carrying the R334W substitution suggesting a spontaneous mutation underlying early-onset sarcoid arthritis.

CASE REPORT

Our patient, a young Caucasian boy, developed a papular rash over his right shoulder at the age of 6 months, which resolved with topical corticosteroids. Soon after, a similar exanthem developed on the right thigh and then generalized. At ages 12 and 15 months 2 skin biopsies confirmed the presence of noncaseating granulomas. At age 20 months he developed symmetrical polyarthritis of the mid-tarsal joints, knees, proximal interphalangeal joints (PIP), and radiocarpal joints associated with morning stiffness. At age 25 months an arthroscopic biopsy of the right knee showed chronic synovitis with noncaseating granulomas. At the age of 5 years he developed bilateral anterior uveitis.

He had normal prenatal and perinatal histories.

His 2 nonconsanguineous parents and his younger brother have remained healthy, with no evidence of any rheumatic or ocular disease despite 20 years of followup. A sister died at age 1 year of complications of cystic fibrosis.

Initial evaluation at our center at the age of 39 months revealed a well appearing young boy tracking at the 25th percentile for weight and height, who was normotensive and afebrile. He had a typical tan-colored erythematous scaly rash mainly on his legs, shoulders, and trunk. He had a moderate Cushingoid appearance and his cardiopulmonary, abdominal, neurological, and peripheral vascular examinations were normal. He had limitation of neck motion, boggy synovial thickening of both wrists with 130° range of motion, remarkable swelling of all PIP joints of both hands and swelling and limitation of both elbows, slight hip limitation, bilateral ankle boggy synovitis, and tenderness over 1st to 4th metatarsal phalangeal (MTP) joints bilaterally. His initial laboratory evaluation revealed hemoglobin of 10.4 g%, white blood cell count 7400 mm^3, platelets 386,000 mm^3, erythrocyte sedimentation rate 34 mm/h, AST 33 IU/l (normal 25–41), ALT 8 IU/l (3–36), blood urea nitrogen 10 mg%, normal urinalysis, angiotensin converting enzyme (ACE) 48 U/l (normal 8–52), and anti-Lyme antibodies were absent. In a previous evaluation at 11 months of age his ANA was negative, complement (C3) level normal at 95 U/ml, ACE 41 U/l (12–36), and rheumatoid factor was absent. The skin specimen was described as a granulomatous infiltrate involving the whole thickness of the dermis, with some perifollicular predominance and abundant multinucleated giant cells of the foreign-body type. Alcian blue and acid-fast bacillus stains were negative. A synovial fluid sample showed 9750 cells/mm^3 with 23% polymorphonuclear neutrophils, 61% lymphocytes, and 16% monocytes. His chest radiographs were normal and the skeletal radiographs showed periarticular osteopenia at the affected locations as well as prominent soft tissue swelling.

The articular course was characterized by corticosteroid dependency with unremitting polyarthritis. Deformities, however, were limited to contracture of the PIP joints and distal radioulnar subluxation. At age 18 years he was eventually tapered to 10 mg every other day of prednisone as a result of infliximab therapy (5 mg/kg/dose intravenously every 8 weeks). He is attending higher education and working part-time. His uveitis after 20 years of disease has remained active. At age 10 he was noted to have normal lenses and pressures and some vitreal involvement, with no evidence of retinitis. He required topical corticosteroids and occasional subconjunctival corticosteroid injections. At age 13 his vision was 20/40 on the right and 20/50 on the left. He underwent bilateral peripheral vitrectomy, with good results. Due to secondary cataract he required lensectomy on the left at age 14. Currently his uncorrected vision is 20/30 on the right and 20/60 on the left. There was no evidence of anterior chamber inflammation at his most recent evaluation.

Genetic testing. The patient agreed with informed consent to participate in genetic research and provided a cheek swab sample as a source of DNA. Genomic DNA was extracted from cheek cells using the Puregene DNA purification kit according to manufacturer's instructions (Gentra Systems, Minneapolis, MN, USA). Polymerase chain reaction (PCR) was performed to amplify a region of exon 4 from *CARD15* using FastStart Taq DNA polymerase with GC-RICH Solution (Roche Diagnostics, Mannheim, Germany) and a touchdown PCR strategy. Since the yield and purity of genomic DNA did not allow a robust PCR amplification, a secondary PCR amplification of the first reaction product was performed using an internal primer set. The resultant PCR product was excised from an agarose gel, purified using Zymoclean Gel DNA Recovery Kit (Zymo Research, Orange, CA, USA), then subjected to direct DNA sequencing in both directions on an ABI377 automated fluorescence DNA sequencer (Applied Biosystems, Foster City, CA, USA) by the Portland VA Medical Center Molecular Biology Core Laboratory.

Results. The majority of *CARD15* exon 4 (i.e., corresponding to mRNA nucleotides 752–2407) was sequenced in both directions from the secondary PCR amplification product. Sequencing revealed a C to T mutation at position 1105, a mutation reported previously in Blau syndrome families. The mutation causes a substitution of tryptophan for arginine at amino acid position 334 (R334W). In addition to this mutation, we detected the presence of 3 other polymorphisms in exon 4: one corresponds to an amino acid substitution of proline to serine at position 268 (P268S) and the other 2 are silent mutations, encoding arginine at position 459 (R459R) and arginine at position 587 (R587R). All these polymorphisms are common in the general Caucasian population, and are not considered associated with disease. Mutation studies of the parents and unaffected brother of the patient are under way.

DISCUSSION

We describe what we believe is the first case of nonfamilial childhood granulomatous arthritis carrying one of the *CARD15* mutations described for Blau syndrome. These mutations are seen in only 50% of affected members of Blau pedigrees[7,8], suggesting that either alternative mutations exist or there is locus heterogeneity. The latter is possible for both conditions (sporadic and familial) since they are defined on the basis of clinical and histologic features. Miceli-Richard, *et al* did not find a mutation in *CARD15* from the 2 sporadic cases tested[7], although one patient had alveolitis, a manifestation not usually reported with either Blau syndrome or early-onset (childhood) granulomatous arthritis.

Blau syndrome has been described as a fixed triad of synovitis, uveitis, and rash with autosomal dominant inheritance[4,12,13], thus excluding a number of reported families showing in addition diverse forms of visceral involvement. Manouvrier-Hanu, *et al*[14] have suggested a continuum model instead for the clinical manifestations of Blau syndrome. At one end is the so-called typical subset with involvement of skin, joints, and eyes and at the other a systemic form where the vascular involvement is severe (familial juvenile systemic granulomatosis). They also defined an

intermediate group and called it atypical Blau syndrome — with arthritis, rash, and uveitis plus one additional territory of involvement. The intermediate group would include our own reported patient with liver granulomas[6] and the family described by Jabs, *et al*[3]. The "systemic granulomatosis" group encompassed the families reported by Rotenstein, *et al*[15] and by Hafner and Vogel[16]. These families had severe arteritis, fever, and malignant hypertension. The frequency of mutations in the intermediate and severe groups is not known, but the family we described[6], with at least one member showing liver granuloma, has subsequently been tested and shown to have substitution R334Q (manuscript in preparation).

The sporadic group of childhood granulomatous arthritis is less understood. Gross, *et al* describe a young girl with childhood sarcoidosis and documented renal artery stenosis[17], and we reported a case of a young girl with Takayasu-like arteritis and childhood sarcoidosis[18]. Of interest, that patient delivered a child later who developed polyarthritis and rash, changing the classification of that family to Blau syndrome. Their mutation studies are still under way.

Based only on clinical data, Miller predicted 18 years ago that neither the extent of the involvement nor the presence or absence of family history are strong enough to classify these 2 entities separately[5]. Our observation of the presence of the mutation in one individual with a sporadic disease (his family members are being tested) and in one family with "atypical Blau syndrome"[6] starts to lend biological substantiation to an extensive body of clinical observations suggesting a single-disease entity. Miller suggested renaming this condition "juvenile systemic granulomatosis"[5] with diverse organ involvement and sporadic and familial forms. This proposition should be reviewed and the search for other mutations intensified.

Added in proof:
A recent report describes a case consistent with Blau syndrome in which the patient was found to have the *CARD15* mutation encoding the R334W amino acid substitution (Kanazawa N, et al. J Invest Dermatol 2004;122:851-2).

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