

Letter to the Editor

Novel presentation of Omenn syndrome in association with aniridia

To the Editor:

Omenn syndrome (OS) is an autosomal recessive combined immunodeficiency characterized by infiltration of the skin and gastrointestinal tract by activated oligoclonal T lymphocytes.¹ Patients are profoundly hypogammaglobulinemic, with few or absent circulating B lymphocytes but high IgE levels. The most common causes of OS are hypomorphic mutations of the recombination-activating genes (*RAG*).¹ Mutations of the *Artemis* (*DCLRE1*), IL-7 receptor α , *RMRP*, IL-2 receptor γ , and *CHD7* genes can also result in the OS phenotype. *RAG1* and *RAG2* are located on chromosome 11p13 in close proximity to a cluster of genes that are deleted in microdeletion 11p13 syndrome. This syndrome, also known as Wilms tumor susceptibility, aniridia, genitourinary abnormalities, and mental retardation (WAGR), arises from deletions encompassing paired box gene 6 (*PAX6*) and Wilms tumor 1 gene (*WT1*). *PAX6* encodes a transcription regulatory protein and is essential for the development of multiple tissues in the eye, including the iris, lens, and neuroretina. Heterozygous *PAX6* mutations often cause hereditary aniridia.² Additionally, *PAXNEB* (a *PAX6* neighbor gene) mutations on 11p13 have been studied for a possible association with aniridia.

We describe a novel presentation of OS associated with aniridia. We show evidence that this unique phenotype results from compound heterozygosity for *RAG* point mutations on the maternal allele along with a contiguous deletion encompassing both *RAG* and *PAX6* genes on the corresponding region of the paternally derived chromosome 11.

The patient was a 3-month-old boy admitted for treatment of a purulent pericardial effusion with blood cultures positive for *Achromobacter xylosoxidans*. Previously, he had been a full-term infant with aniridia who had an erythematous peeling rash on his entire body during the first week of life. This was treated as eczema, with temporary improvement followed by progressive worsening. He experienced poor weight gain through his first 3 months. He was the only child of nonconsanguineous healthy parents of Western European descent. The family history was negative both for immunodeficiency and aniridia.

The physical examination was notable for severe erythema with diffuse skin peeling, seborrheic dermatitis, and paucity of hair. Aniridia was noted. A 2/6 holosystolic murmur was present. The rest of the physical examination was normal. Of note, there were no genital abnormalities, lymphadenopathy, or organomegaly. Initial laboratory studies included a white blood cell count of 81,970 cells/ μ L with 15% segmented neutrophils, 73% lymphocytes, 8% eosinophils, and 2% basophils; a hemoglobin count of 9.1 g/dL; a hematocrit value of 30%; a platelet count of $263 \times 10^3/\mu$ L; an IgG level of 41 mg/dL; an IgA level of 9 mg/dL; an IgM level of 8 mg/dL; and an IgE level of 196 mg/dL. Additional studies revealed an absolute lymphocyte count of 59,840 cells/ μ L, an absolute CD3⁺ count of 50,226 cells/ μ L (96%), an absolute CD4⁺ count of 18,737 cells/ μ L (32%), an absolute CD8⁺ count of 36,142 cells/ μ L (62%), an absolute CD19⁺ count of 37 cells/ μ L (0%), and an absolute CD16⁺/CD56⁺ count of 1,107 cells/ μ L (2%). The patient's lymphocytes were unresponsive to PHA, concanavalin A, and pokeweed mitogen. His

karyotype was normal 46, XY. Maternal engraftment was excluded by means of fluorescent *in situ* hybridization. PCR amplification of lymphocyte V γ -J γ segments showed oligoclonality with profoundly limited T-cell diversity, which is consistent with OS (Fig 1). The presenting laboratory evaluation was consistent with OS. The initial white blood cell and absolute lymphocyte counts are relatively high and might be reflective of coexistent infections but are within published ranges for OS.³ The IgE level, although not markedly increased, is also in the range reported for OS.

DNA sequencing identified 2 apparently homozygous missense mutations in *RAG1* (p.Met435Val and p.Met1006Val) and 1 apparently homozygous missense mutation in *RAG2* (p.Met502Val). The p.Met435Val mutation in *RAG1* and the p.Met502Val mutation in *RAG2* have previously been reported in OS.^{4,5} Chromosomal microarray analysis revealed a deletion of approximately 9.5 Mb extending from 11p12 to 11p14.1. This deletion encompassed both *RAG* genes, *WT1*, and *PAX6*. The mother was heterozygous for the same 3 missense mutations on *RAG1* and *RAG2*. The father's sequences were normal. Paternal DNA was unavailable for further analysis, but the absence of aniridia in the father along with the large size of the deletion provide strong evidence that it arose *de novo*.

Taken together, these data indicate that the *RAG1* and *RAG2* missense mutations identified in the proband lie in *cis* on the non-deleted maternally inherited chromosome, whereas a deletion encompassing *RAG1/RAG2/PAX6* arose *de novo* on the paternally inherited chromosome. This combination results in a partial loss of *RAG1/RAG2* function and consequent OS. The heterozygous deletion of the *PAX6* gene is responsible for the aniridia (Fig 2). This contiguous deletion also includes the *WT1* gene. With the exception of aniridia, our patient has not yet manifested any other features of microdeletion 11p13 (WAGR) syndrome. He has been evaluated for genitourinary malformations and is being closely monitored for Wilms tumor.

After clearance of infection, the patient underwent bone marrow transplantation from a 10/10 matched unrelated donor after myeloablative conditioning with busulfan, cyclophosphamide, and antithymocyte globulin. He is doing well off of immunosuppression, with no evidence of graft-versus-host disease 1 year later.

This represents the first report of OS arising from a combination of a mutation and deletion with resultant loss of function and represents a novel clinical presentation of aniridia in association with OS. One report of several patients with aniridia caused by large deletions mentioned a single patient who also had immunodeficiency but with no specific details.⁶ Deletions on chromosome 11 are a well-documented cause of aniridia,^{6,7} but the connection between aniridia and immunodeficiency has not been recognized explicitly. Because deletions in this region are not uncommon, this case underscores the importance of testing for large deletions to assess the risk of *WT1* deletion in patients presenting with OS-related immunodeficiency in whom genetic analysis suggests apparent homozygosity and in whom parental sequencing does not establish inheritance of both maternal and paternal loss-of-function *RAG* alleles. A deletion could include the *RAG* genes and the *WT1* gene and not be obvious clinically if the *PAX6* gene were not deleted. Additionally, screening for

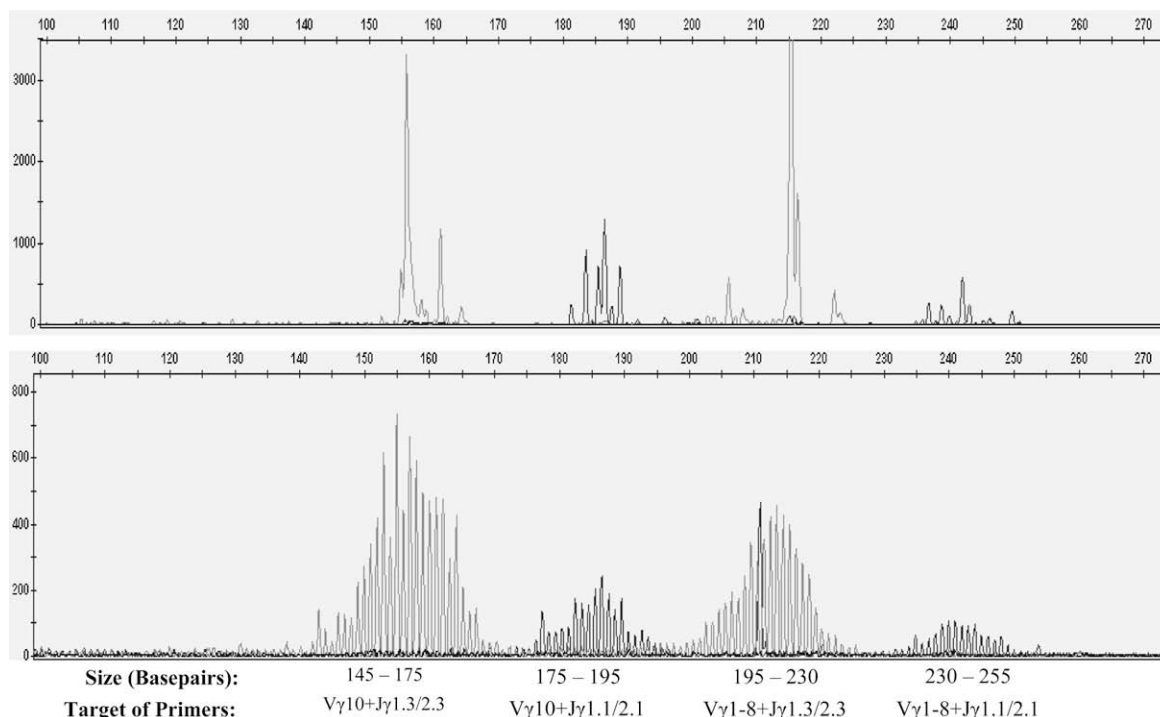


FIG 1. T-cell receptor γ gene rearrangement clonality analysis. Multiplex PCR amplification with primers to the variable and joining regions of T-cell receptor γ was used to evaluate the V-D-J rearrangements for diversity. PCR product sizes are shown on the x-axis, and peak fluorescence is shown on the y-axis. *Top row* (patient with OS): Oligoclonal pattern indicative of limited TCR γ diversity. *Bottom row* (control subjects): Normal distribution of T-cell receptor γ rearrangement indicative of adequate diversity.

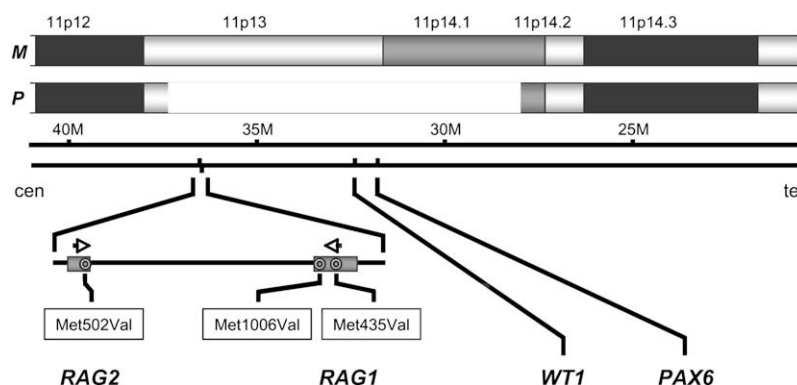


FIG 2. Schematic representation of chromosome 11p with the deletion and *RAG1/RAG2* gene mutations (circles) in the patient's paternally (*P*) and maternally (*M*) derived alleles. The deletion encompasses approximately 9.5 Mb, extending from 11p12 to 11p14.1 (chr11:27,956,661-37,509,625; hg18). The median spacing of probes on the microarray is 9 kb.

immunodeficiency in patients with aniridia caused by a deletion becomes a consideration. Screening could be accomplished with a complete blood count with differential, lymphocyte subsets, and immunoglobulin levels. Any results consistent with OS would prompt further genetic testing. In these cases detailed deletion analysis, sequencing, or both of the *RAG* genes would be indicated because coincident inheritance of a *RAG1* or *RAG2* mutation would be the only way that OS would arise in conjunction with microdeletion 11p13 syndrome.

A number of other primary immunodeficiencies exhibit autosomal recessive inheritance and as such require abnormality in

both alleles for phenotypic expression. It is predictable that any of these diseases might result from a heterozygous mutation complemented by a deletion. This scenario has previously been reported for the gene encoding Artemis protein in which small deletions encompassing several exons result in loss of function and severe combined immunodeficiency (SCID).⁸ Additionally, adenosine deaminase-deficient SCID has been reported to be caused by gene deletion in 14% of cases.⁹ However, the gross deletion of multiple contiguous genes, compounded by point mutations and leading to a recessive phenotype, has not been reported previously. To date, the available clinical genetic testing for SCID

is primarily sequence-based. Our findings and the reports of other cases of autosomal recessive SCID in which a deletion was found on 1 allele suggest that microarray analysis or other focused deletion testing might be considered if sequencing only reveals mutations on 1 allele.

We thank Yiping Shen and Bai-Lin Wu for their assistance while working in the Department of Laboratory Medicine at Children's Hospital Boston, Boston, Mass.

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W.J.S. is supported by a National Institutes of Health National Research Service Award grant (T32-AI-007512).

Disclosure of potential conflict of interest: D. T. Miller is a consultant for Correlagen Diagnostics and Athena Diagnostics. F. A. Bonilla is a speaker for CSL Behring, Inc, and a consultant for Prescription Solutions, Inc; receives grant support from Talecris Biotherapeutics; and is on the Medical Advisory Committee for the Immune Deficiency Foundation. S. Giliani received grant support from the Italian Ministry

of Research. S.-Y. Pai receives grant support from the Charles M. Hood Foundation. L. D. Notarangelo receives grant support from Children's Hospital Boston and is a member of the Steering Committee for USIDNET and a member of the Immune Disease Institute. H. C. Oettgen is a consultant for Schering-Plough and Genentech and receives grant support from Novartis Pharmaceuticals. The rest of the authors have declared that they have no conflict of interest.

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Available online ■■■.
doi:10.1016/j.jaci.2008.12.007