Published in final edited form as:

J Allergy Clin Immunol. 2009 April ; 123(4): 966–969. doi:10.1016/j.jaci.2008.12.007.

Novel Presentation of Omenn Syndrome in Association with Aniridia

William J. Sheehan, MD^{a,b}, Ottavia M. Delmonte, MD^{a,b,c}, David T. Miller, MD, PhD^{b,d}, Amy E. Roberts, MD^{b,d}, Francisco A. Bonilla, MD, PhD^{a,b}, Massimo Morra, MD, PhD^e, Silvia Giliani, PhD^f, Sung-Yun Pai, MD^{b,g,h}, Luigi D. Notarangelo, MD^{a,b}, and Hans C. Oettgen, MD, PhD^{a,b}

a Division of Immunology, Children's Hospital, Boston, MA, 02115, USA

b Harvard Medical School, Boston, MA, 02115, USA

c Ospedale Regina Margherita, Università di Torino, Torino, Italy

d Division of Genetics, Children's Hospital, Boston

e Correlagen Diagnostics, Waltham, MA, 02452, USA

f Angelo Nocivelli Institute of Molecular Medicine, University of Brescia, Brescia, Italy

g Division of Hematology-Oncology, Children's Hospital, Boston

h Dana-Farber Cancer Institute, Department of Pediatric Oncology, Boston, MA, 02115, USA

Summary

We report a case of Omenn Syndrome presenting in association with aniridia arising from 3 maternally-inherited RAG mutations compounded by a deletion encompassing *RAG* and *PAX6* genes on the paternally-inherited chromosome.

Keywords

Omenn Syndrome; Aniridia; Recombination-Activating Genes; PAX 6; Wilm's Tumor; Immunodeficiency

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 *Artemis* (*DCLRE1*), *IL7RA*, *RMRP*, *IL2RG*, and *CHD7* genes may also result in the OS phenotype. *RAG1* and *RAG2* are

Correspondence: Hans C. Oettgen, MD, PhD, Division of Immunology, Children's Hospital Boston, 300 Longwood Avenue, Boston, MA, 02115, Phone: (617) 919-2488 Fax: (617) 730-0310, Email: E-mail: Hans.Oettgen@childrens.harvard.edu.

Disclosure of potential conflict of interest: Massimo Morra is employed by Correlagen Diagnostics.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

located on chromosome 11p13 in close proximity to a cluster of genes which are deleted in Microdeletion 11p13 Syndrome. This syndrome, also known as WAGR (Wilms tumor susceptibility, Aniridia, Genitourinary abnormalities, and mental Retardation), arises from deletions encompassing *PAX6* and *WT1* genes. *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* (*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* genes and *PAX6* on the corresponding region of the paternally-derived chromosome 11.

The patient was a 3-month-old male 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 developed 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 exam 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 exam was normal. Of note, there were no genital abnormalities, lymphadenopathy, or organomegaly. Initial lab studies included: WBC 81,970 cells/ μ L with 15% segmented neutrophils, 73% lymphocytes, 8% eosinophils, and 2% basophils; hemoglobin 9.1 g/dL; hematocrit 30%; platelet count $263 \times 10^3/\mu$ L; IgG 41 mg/dL; IgA 9 mg/dL; IgM 8 mg/dL; and IgE 196 mg/dL. Additional studies revealed an absolute lymphocyte count of 59,840 cells/ μ L, absolute CD3+ 50,226 cells/ μ L (96%), absolute CD4+ 18,737 cells/ μ L (32%), absolute CD8+ 36,142 cells/ μ L (62%), absolute CD19+ 37 cells/ μ L (0%), and absolute CD16+/CD56+ 1,107 cells/ μ L (2%). The patient's lymphocytes were unresponsive to phytohemagglutinin, concanavalin A, and pokeweed mitogen. His karyotype was normal 46, XY. Maternal engraftment was excluded by fluorescent *in situ* hybridization (FISH). PCR amplification of lymphocyte V γ – J γ segments showed oligoclonality with profoundly limited T-cell diversity consistent with OS. (See Figure 1) The presenting laboratory evaluation was consistent with OS. The initial WBC and ALC are relatively high and may be reflective of coexistent infections, but are within published ranges for OS.³ The IgE level, although not markedly elevated 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*. Mother was heterozygous for the same 3 missense mutations on *RAG1* and *RAG2*. 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 while 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. (See Figure 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 anti-thymocyte globulin. He is doing well off of immunosuppression with no evidence of graft vs. host disease one 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 due to 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. As 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 Omenn syndrome-related immunodeficiency in whom genetic analysis suggests apparent homozygosity and where 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 immunodeficiency in patients with aniridia due to 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 and/or sequencing of the *RAG* genes would be indicated, since 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, ADA 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 one allele suggest that microarray analysis or other focused deletion testing might be considered if sequencing only reveals mutations on one allele.

Acknowledgments

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, MA.

Declaration of funding: W.J. Sheehan is supported by an NIH NRSA grant (T32-AI-007512).

Abbreviations

OS

Omenn Syndrome

RAG

Recombination-Activating Genes

WT1

Wilms Tumor 1 Gene

WAGR

Wilms tumor susceptibility, Aniridia, Genitourinary abnormalities, and Mental Retardation

FISH

Fluorescent In Situ Hybridization

References

1. Villa A, Santagata S, Bozzi F, Giliani S, Frattini A, Imberti L, et al. Partial V(D)J recombination activity leads to Omenn syndrome. *Cell* 1998;93:885–96. [PubMed: 9630231]
2. Davis A, Cowell JK. Mutations in the PAX6 gene in patients with hereditary aniridia. *Hum Mol Genet* 1993;2:2093–7. [PubMed: 8111379]
3. Aleman K, Noordzij JG, de Groot R, van Dongen JJ, Hartwig NG. Reviewing Omenn syndrome. *Eur J Pediatr* 2001;160:718–25. [PubMed: 11795679]
4. Villa A, Sobacchi C, Notarangelo LD, Bozzi F, Abinun M, Abrahamsen TG, et al. V(D)J recombination defects in lymphocytes due to RAG mutations: severe immunodeficiency with a spectrum of clinical presentations. *Blood* 2001;97:81–8. [PubMed: 11133745]
5. Haq IJ, Steinberg LJ, Hoenig M, van der Burg M, Villa A, Cant AJ, et al. GvHD-associated cytokine polymorphisms do not associate with Omenn syndrome rather than T-B- SCID in patients with defects in RAG genes. *Clin Immunol* 2007;124:165–9. [PubMed: 17572155]
6. Crolla JA, van Heyningen V. Frequent chromosome aberrations revealed by molecular cytogenetic studies in patients with aniridia. *Am J Hum Genet* 2002;71:1138–49. [PubMed: 12386836]
7. Robinson DO, Howarth RJ, Williamson KA, van Heyningen V, Beal SJ, Crolla JA. Genetic analysis of chromosome 11p13 and the PAX6 gene in a series of 125 cases referred with aniridia. *Am J Med Genet A* 2008;146A:558–69. [PubMed: 18241071]
8. Moshous D, Callebaut I, de Chasseval R, Corneo B, Cavazzana-Calvo M, Le Deist F, et al. Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human severe combined immune deficiency. *Cell* 2001;105:177–86. [PubMed: 11336668]
9. Vihinen M, Arredondo-Vega FX, Casanova JL, Etzioni A, Giliani S, Hammarstrom L, et al. Primary immunodeficiency mutation databases. *Adv Genet* 2001;43:103–88. [PubMed: 11037300]

Figure 1.

TCR γ gene rearrangement clonality analysis. Multiplex PCR amplification using primers to the variable and joining regions of TCR γ was used to evaluate the V-D-J rearrangements for diversity. PCR product sizes are shown on the x-axis and peak fluorescence on the y-axis.

Top Row (OS Patient): Oligoclonal pattern indicative of limited TCR γ diversity.

Bottom Row (Control): Normal distribution of TCR γ rearrangement indicative of adequate diversity.

Figure 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.