

Mutations in the RNA component of RNase mitochondrial RNA processing might cause Omenn syndrome

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Background: Omenn syndrome is a variant of severe combined immunodeficiency disease, which most prominently presents with erythroderma, eosinophilia, and susceptibility to various pathogens. Mutations in the nucleases of recombination activating genes 1 and 2 (*RAG1/RAG2*) or *Artemis* were found in some, but not all, patients with Omenn syndrome. We identified 2 patients who presented with clinical features consistent with Omenn syndrome but had no mutations in *RAG* or *Artemis*. Both patients also had cartilage-hair hypoplasia (CHH).

Objectives: We sought to define the molecular basis and characterize the features of severe combined immunodeficiency and Omenn syndrome in these patients.

Methods: We have studied humoral and cellular immunity using standard assays. T-cell repertoire was investigated by quantitating V β families. The RNase mitochondrial RNA processing (*RMRP*) RNA gene was sequenced by using standard techniques.

Results: Sequence analysis of the *RMRP* RNA gene showed that each patient had an insertion-duplication on one allele and a point mutation on the other allele. These point mutations were novel, and they might be related to the unusual presentation of Omenn syndrome in addition to CHH in these patients. Indeed, analysis of the thymus showed residual mature T lymphocytes. This leaky thymus might be responsible for the skewed release of some T-cell clones into the circulation, which might trigger the phenotype of Omenn syndrome.

Conclusion: We have demonstrated that mutations in the *RMRP* RNA gene might be associated with Omenn syndrome. Clinical implications: This discovery will aid clinicians in the early recognition and treatment of CHH-associated Omenn syndrome. (*J Allergy Clin Immunol* 2006;117:897-903.)

Key words: Primary immunodeficiency, mutation

Abbreviations used

BMT:	Bone marrow transplant
CHH:	Cartilage-hair hypoplasia
MRP:	Mitochondrial RNA processing
RAG:	Recombination activating gene
RMRP:	RNase mitochondrial RNA processing
SCID:	Severe combined immunodeficiency

Omenn syndrome is a severe combined immunodeficiency (SCID) typically presenting in infancy with a generalized scaly exudative erythroderma, enlarged lymphoid tissues, protracted diarrhea, failure to thrive, and eosinophilia. This is a fatal condition unless treated with bone marrow transplantation.^{1,2}

Treatment with cyclosporine, corticosteroids, or both before bone marrow transplantation results in the amelioration of skin inflammation and the regression of lymphadenopathy.³ This is believed to be due to suppression of oligoclonal T cells, which secrete T_H2-type cytokines.⁴⁻⁶ Indeed, many studies consistently showed that T-cell repertoire in Omenn syndrome is severely restricted, with one or several clones markedly expanded.^{3,7}

The skewed T-cell representation can be explained and is frequently caused by mutations in the recombination activating genes 1 or 2 (*RAG1* or *RAG2*).⁸ These enzymes play a critical role in the complex process of assembling the V, (D), and J segments, which together form the variable segments of immunoglobulin and T-cell receptor protein chains.⁹⁻¹² In the absence of effective recombination, the development of a full repertoire of T cells and B cells is limited. In fact, null mutations of *RAG1* or *RAG2* genes cause SCID with an absence of mature T and B cells.¹³ Mutations in *RAG1/RAG2* with residual enzymatic activity tend to cause Omenn syndrome with a variety of phenotypic presentations.^{8,14} Although some patients present with all or most of the classical features, others might display only a few manifestations (atypical).^{7,8,14}

In a similar fashion, complete lack of another nuclease, *Artemis*, leads to a block in T- and B-cell development and to SCID.^{15,16} When associated with DNA-dependent protein kinase, *Artemis* exhibits endonuclease and DNA hairpin-opening activities.¹⁷ Recently, a partial deficiency of *Artemis* was shown to cause Omenn syndrome, likely by allowing partial V(D)J recombination similar to partial *RAG1/RAG2* deficiency.¹⁸

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TABLE I. Clinical features

	Patient 1	Patient 2
General features		
Age (mo)	5	3
Sex	F	F
Outcome	Alive and well 10 years after BMT	Alive and well 15 years after BMT
Omenn features		
Skin and mucus membranes	Severe erythroderma, oral thrush	Mild erythroderma
Lymph nodes	Lymphadenopathy	Lymphadenopathy
Liver	Enlarged (4 cm)	Enlarged (2 cm)
Spleen	Enlarged (3 cm)	Enlarged (4 cm)
Lungs	<i>Pneumocystis carinii</i>	<i>Pneumocystis carinii</i>
GI	Rotavirus, protracted diarrhea	Protracted diarrhea
Growth		
Height (<1y)*	<3rd	<3rd
Weight (<1y)*	<3rd	<3rd
Height (current)*	<3rd	<3rd
Weight (current)*	25th	50th
Diagnosis of CHH (y)	3	4

GI, Gastrointestinal tract; <1y, less than 1 year of age.

*Percentile.

However, not all cases of Omenn syndrome harbor mutations in *RAG* or *Artemis* genes.¹⁹ In our center mutations in these genes have only been identified in less than 50% of patients with Omenn syndrome, clearly indicating that mutations in other genes can cause Omenn syndrome.

We show here for the first time that mutations in the RNA component of the RNase mitochondrial RNA processing (*RMRP*) RNA gene might cause Omenn syndrome. This gene is the only known nuclear gene that encodes the RNA component of the *ribonuclease mitochondrial RNA processing* (*MRP*) gene.²⁰ The RNA encoded by this gene is not translated into protein but is associated with the large protein RMRP to form a complex capable of cleaving the primer RNA needed for mitochondrial DNA replication and of processing 5.8S ribosomal RNA.²¹ Mutations in this gene have been previously shown to be associated with the skeletal dysplasia cartilage-hair hypoplasia (CHH).²²⁻²⁵ CHH was first described by McKusick et al²⁶ as a metaphyseal chondrodysplasia in the Amish. This short-limb dwarfism might be associated with cellular immunodeficiency and occasionally with humoral defects.^{27,28} Rarely, patients with CHH have profound T-cell deficiency indistinguishable from SCID, leading to fatal varicella and necessitating a bone marrow transplantation.^{2,29}

METHODS

Cellular and humoral evaluation

Lymphocyte markers have been evaluated by using flow cytometry, as previously shown.^{30,31} Proliferative responses to mitogens and anti-CD3 antibody were performed with standard methods.^{30,31}

IgG, IgM, and IgA levels were measured on the basis of nephelometry, whereas IgE levels were detected with an RIA kit.^{30,31} (For details, see the online-only [Methods](#) section in the Online Repository at www.jacionline.org.)

Quantitation of T-cell receptor V β genes and analysis of the RMRP RNA gene and its transcripts

Specific T-cell receptor V β mRNA specimens were quantified by means of PCR with synthetic DNA as an internal standard, as previously described.^{4,32,33}

Genomic DNA derived from patients' lymphocytes was analyzed by means of PCR amplification and direct sequencing of the RMRP RNA gene.²¹ For detection of allelic mutations, the PCR fragments were cloned into TA vector, and single colonies were picked and sequenced with the primers 5'-CTGAGAATGAGCCCGTGTGGTTGG-3', 5'-CAGCCGCGCTGAGAATGAGCC-3', 5'-TGCTGAAGGCCTGTATCC-3', and 5'-CTAGAGGGAGCTGACGGATGACG-3'. Northern blot analysis of the RMRP RNA was done as previously described.³³

RESULTS

Patients

Case 1. Born at term after a normal pregnancy and delivery, the 5-month-old patient had severe failure to thrive after a prolonged episode of bronchiolitis and chronic diarrhea. She was subsequently admitted to the hospital for deteriorating respiratory distress and oral thrush, which was resistant to treatment with nystatin. On examination, she had lymphadenopathy, as well as an enlarged spleen and liver 3 and 4 cm below the costal margin, respectively. She had severe erythroderma, with breakdown of the skin in multiple areas. The skin condition predisposed her to abscess formation in the left leg, as well as the abdominal wall (Table I).

Bronchial secretions yielded the isolation of *Pneumocystis carinii*, and a stool specimen contained rotavirus. Antibiotic therapy improved her lung and skin abscesses, but her diarrhea and rash worsened. At this stage, she was given the diagnosis of Omenn syndrome. Over the ensuing several months, her skin rash deteriorated, lymphadenopathy became more prominent, and the size of the liver and spleen increased. She was then treated with 2 mg/kg prednisone, with marked improvement of the skin condition and reduction in the size of lymphoid tissues. She subsequently received a haploidentical bone marrow transplant (BMT), which failed. She then received a successful matched unrelated BMT. She is alive and well almost 10 years after transplantation (Table I).

Case 2. This patient was born at term after a normal pregnancy and delivery to nonconsanguineous parents of English descent. She had diarrhea at 3 months of age, and her weight gain was poor. At the age of 8 months, she had *Pneumocystis carinii*-induced pneumonitis, which responded well to treatment with high-dose Septra (Monarch Pharmaceuticals, Bristol, Tenn). On this admission, she was noticed to have a prominent skin rash on the face trunk and generalized erythroderma. Enlarged

inguinal and axillary lymph nodes were palpated bilaterally. Smaller-size nodes were also detected in occipital and retroauricular areas.

A diagnosis of T-cell immunodeficiency (Omenn syndrome) was made at the age of 10 months on the basis of poor T-cell mitogenic responses, negative *Candida* species skin test results, and a dysplastic thymus. Consequently, the patient received a matched BMT from her male sibling. Full immune reconstitution and rapid weight gain ensued. However, linear growth remained below expected values. She remains fully engrafted and alive and well 15 years after bone marrow transplantation (Table I).

Evaluation of the immune system

Both patients were persistently lymphopenic, and patient 1 periodically had reduced neutrophil counts. Flow cytometric analysis showed in both patients markedly reduced CD3⁺ T cells, whereas CD20⁺ B cells were normal. CD56⁺ natural killer (NK) cells were normal in patient 1 but reduced in patient 2. Proliferative responses to PHA were markedly decreased, and so were the responses to anti-CD3 antibody (Table II). Addition of exogenous IL-2 did not increase the proliferative response, and neither did the incubation of the patient's mononuclear cells with the combination of phorbol esters and the Ca²⁺ ionophore ionomycin (not shown). This suggested that the defect was not likely to involve early signaling events. The results of *in vivo* *Candida* species skin testing were negative in both patients, suggesting a profound T-cell immunodeficiency.

The humoral immunity was also affected because patient 1 had borderline low serum IgG levels and undetectable IgA or IgM levels. Patient 2 had normal serum IgG and IgM levels but undetectable IgA levels. Specific antibodies in response to vaccines, such as tetanus and polio, were markedly decreased (Table II). Together, these results suggested the patient had SCID.

Clonality of T lymphocytes

One of the hallmarks of Omenn syndrome is the abnormal expansion in peripheral blood and tissues of one or more T-cell clones. Missense mutations in *RAG1*/*RAG2* or *Artemis* might hamper but not completely abolish the function of these enzymes, resulting in a leaky defect,^{14,18} which allows for maturation in the thymus of very limited numbers of T cells.

In a similar fashion the RMRP mutations described thus far do not cause a complete absence of MRP RNA, permitting some activity of the enzyme complex.²²⁻²⁵ We therefore studied the patterns of rearrangement of the T-cell receptor genes in these patients to explore the possibility of a skewed T-cell repertoire. We used quantitative PCR to determine the TCR V β family use in peripheral blood lymphocytes, as previously described.^{4,30,31}

When compared with a control specimen, patient 1 only expressed 50% of V β families tested (Fig 1). Six of them, V β 3, V β 5, V β 10, V β 11, V β 15, and V β 16, were moderately to severely underrepresented. In contrast, V β 12 and V β 14 showed increased representation when compared

TABLE II. Humoral and cellular immunity

	Patient 1	Patient 2	Normal range
Serum immunoglobulins (g/L)			
IgG	5.6	6.2	2.3-14.1
IgM	<0.1	1.12	0.1-1.4
IgA	<0.1	<0.1	0.1-0.8
Specific antibodies			
Tetanus (IU/mL)	0.01	<0.01	>0.04
Polio	<1:8	<1:8	>1:16
Lymphocyte markers (cells/ μ L)			
CD3	340	237	2000-6900
CD4	289	133	1400-5100
CD8	21	142	600-2200
CD19	170	475	700-2500
CD56	980	47	100-1000
Mitogenic responses*			
	Patient 1/ control specimen	Patient 2/ control specimen	
PHA	3/64	14/107	
Anti-CD3	5/80	7/92	

*Mitogenic responses are expressed as the stimulation index.

with controls. These results clearly demonstrate a limited repertoire of T cells in patient 1, as well as the expansion of a limited number of clones.

Evaluation of thymus, lymph nodes, and skin

Sections of thymus biopsy specimens showed that both patients had remarkably identical thymic morphology. Hematoxylin and eosin staining showed a marked depletion of thymocytes and an absence of Hassall's corpuscles, with a complete loss of corticomedullary architecture. The epithelial islands were arranged in small nests of cohesive spindle-shaped cells separated by fibrovascular septa. Immunohistochemical analysis consistently showed an unusual pattern with CD3⁺ thymocytes, which expressed either CD4 or CD8 localized exclusively to the medullary areas (Fig 2).

Sections of lymph node tissue obtained from patient 1 showed a few small primitive lymphoid follicles, some of which possessed primitive germinal centers. The interfollicular zones contained predominantly epithelial cells mixed with eosinophils and reduced numbers of lymphocytes. Immunohistochemical analysis showed depletion of CD3⁺ T cells in the interfollicular areas, which costained with either CD4 or CD8 (not shown).

Sections of the skin showed irregular epidermis infiltrated by lymphocytes in multiple areas. The superficial dermis contained an inflammatory infiltrate consisting predominantly of mononuclear cells and eosinophils. Morphologically the mononuclear cells consisted of mixed populations of lymphocytes, plasma cells, and macrophage-like cells. The infiltrate was most prominent at the dermal-epidermal junction and halfway into the deeper dermis. Immunohistochemistry analysis showed that most mononuclear cells in the superficial dermis were S-100⁺ Langerhans cells. In other areas of the skin, the infiltrates

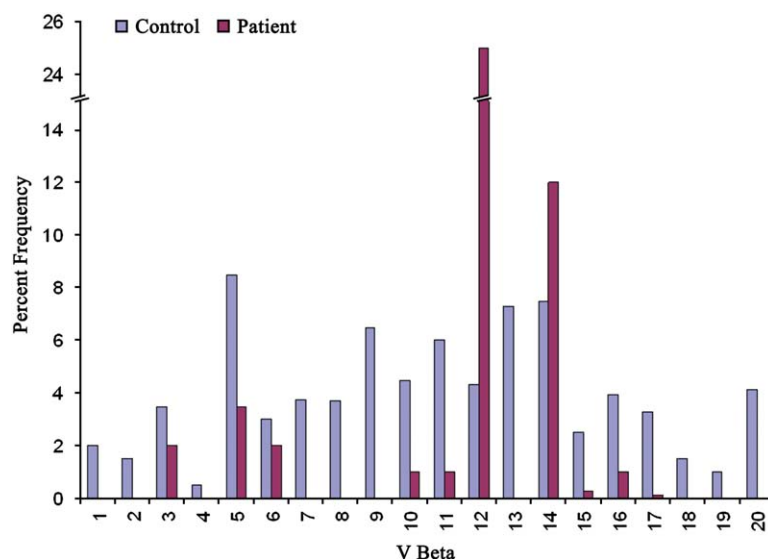


FIG 1. T-cell repertoire: profile of expression of V β families. The amplification of T-cell patient 1 and control RNA through 25 cycles plotted versus the frequency of the 20 different V β families.

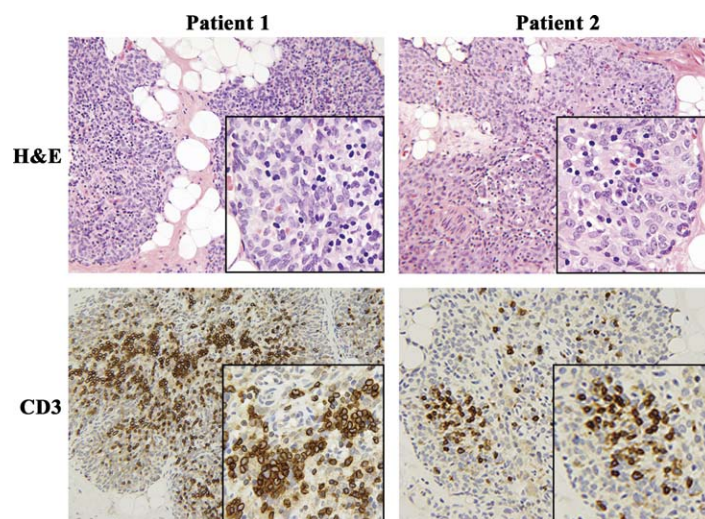


FIG 2. Analysis of thymus histology. Hematoxylin and eosin (H&E) staining (*upper panel*) of thymus tissue obtained from both patients (as indicated) revealed dysplastic changes. There is lack of Hassall's corpuscles and depletion of lymphocytes, with some remaining mononuclear cells in the medullary areas (*enlarged view*). Immunohistochemical analysis with anti-CD3 antibody showed a similar pattern in both patients: a reduced number of CD3⁺ cells confined to the medullary area of the lobule.

consisted mainly of CD3⁺ T cells and only a small number of CD19⁺ B cells. Skin T cells also stained positive for TCR- $\alpha\beta$ and for CD4, whereas staining for CD8 and TCR- $\gamma\delta$ was negative (not shown).

Growth and development

After bone marrow transplantation, patient 1 rapidly recovered and started to gain weight. She also rapidly caught up with developmental milestones in accordance with her age. However, her linear growth remained stunted and was more than 5 SDs below normal range. Patient 2 had a similar course, and her height remained just below

the third percentile. Determination of patient 1's bone age revealed a marked delay. Radiographs of her hand revealed cone-shaped metaphyseal invaginations of the middle and proximal phalanges, as well as the base of the first metacarpal and distal aspects of the third through fifth metacarpal bones. Skeletal survey showed metaphyseal irregularity and sclerosis involving the long bones, which was most marked in the proximal humeri and distal femurs and proximal tibiae, with beaking medially. There were bilateral coxa valga deformities. Views of the spine demonstrate irregularity and beaking anteriorly of the vertebrae, predominantly in the lower thoracic and

TABLE III. Mutation analysis

	Mutation	Polymorphism
Patient 1		
Allele 1:	TACTCTGTGAAGTACTCTG TGAAGCTGA at -10 (insertion)	C-58T T274C G156C C176T
Allele 2:	C4T	
Patient 2		
Allele 1:	ATCTGTG at -13	
Allele 2:	A240C	C-58T C-48A

lumbar regions. Skeletal survey of patient 2 showed irregularities of the distal radial and ulnar metaphyses. Similarly, all the metaphyses of the metacarpals and phalanges were irregular and frayed. Similar changes were noted in the distal and proximal femoral, as well as the tibial and fibular, metaphyses and in the metatarsals and phalanges of both feet. Examination of the spine demonstrated irregular end plates of the vertebral bodies in the thoracolumbar region.

The gradual development over several years of skeletal dysplasia delayed the diagnosis in both patients and was finally made at the ages of 3 and 4 years for patients 1 and 2, respectively. The typical skeletal changes combined with immunodeficiency established the diagnosis of CHH in the patients.

Characterization of the molecular defect

Mutations in the *RMRP* RNA gene have been recently found in patients with CHH. We have therefore reasoned that if *RMRP* mutations can be associated with metaphysical dysplasia and SCID, then genetic aberrations in this gene might also underlie Omenn syndrome. Analysis of the *RMRP* RNA gene was performed on genomic DNA extracted from EBV-transformed B lymphoblasts that were established from the patients' peripheral blood lymphocytes. PCR fragments were cloned into a TA vector to identify mutations on each of the patient's alleles. Fifteen and 13 independent clones from patients 1 and 2, respectively, were subsequently sequenced.

Both patients were compound heterozygotes for *RMRP* RNA mutations. Seven of 13 clones from patient 1 had a 28-bp insertion (including 2 times duplication) in the transcription regulatory region of the *RMRP* gene between the TATA box and the transcription initiation site at position -9 (Table III). The 6 remaining clones, which represent the second allele, carried a novel C4T point mutation. Patient 2 had a 7-nucleotide insertion at position -13 (ATCTGTG) located between the TATA box and the transcription initiation site, which was consistently found in 12 of 15 clones. Three other clones carried another novel A240C mutation. The mother of patient 2 was heterozygous for the insertion, and the father was heterozygous for the point mutation. Other genetic changes previously recognized as polymorphism because of common

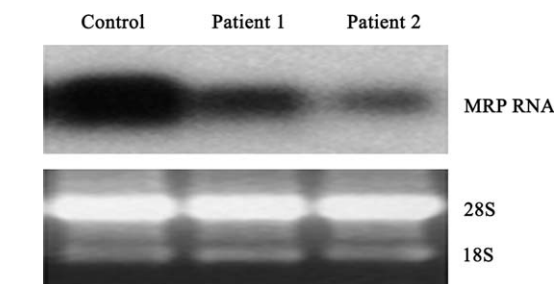


FIG 3. RMRP RNA expression. Northern blot analysis of total RNA from EBV-transformed B lymphoblasts of a healthy control specimen and the patients. Lane 1 represents the healthy control specimen, and lanes 2 and 3 represent patients 1 and 2, respectively.

occurrence in the general population were also detected (Table III).

Insertions in the promoter region of *RMRP* RNA are reported to cause silencing of the corresponding allele.²² Direct sequencing of patient cDNA derived from lymphoblastoid cells showed that only the allele carrying the C4T mutation in patient 1 and the A240C mutation in patient 2 were present, implying that alterations in the promoter region completely ablated expression of these *RMRP* RNA alleles.

RMRP RNA expression in patients' lymphocytes

Northern blot analysis of the *RMRP* RNA revealed expression in both patients (Fig 3). By using the levels of 28S and 18S rRNA as references, *RMRP* RNA was less abundantly present in both patients compared with in a control sample. Quantitative analysis with densitometry has shown that the level of expression of the gene was reduced to 50% in patient 1 and 36% in patient 2 of the level of expression in control cells, respectively.

DISCUSSION

The patients described here had typical features of Omenn syndrome. Both presented with protracted diarrhea, pneumonitis caused by *Pneumocystis carinii*, and universal erythroderma. They subsequently experienced marked lymphadenopathy and hepatosplenomegaly, whereas eosinophilia was prominent in patient 1. They had characteristic low numbers of circulating lymphocytes, which responded poorly to mitogens and to vaccinations. Thymus biopsy in both patients showed dysplastic changes, including thymocyte depletion, loss of corticomedullary architecture, and complete absence of Hassall's corpuscles, which is consistent with the diagnosis of SCID.^{34,35} Poor organization of lymph node architecture was also found in both patients.

Skin biopsy specimens of these patients were compatible with changes observed in other cases of Omenn syndrome. These included heavy mononuclear infiltrates in the dermis, including Langerhans cells, eosinophils, and T cells.⁶ The damage to the skin is believed to be induced

by expansion of oligoclonal T cells that secrete T_H2 -type cytokines.⁴⁻⁶

Similar to other cases of Omenn syndrome and one case of CHH,³⁶ we demonstrate here that patient 1 had a skewed T-cell repertoire, with limited expression of T-cell receptor subfamilies and expansion of $V\beta14$ and $V\beta17$ in peripheral blood. It was assumed, although we could not prove it because of lack of a tissue sample, that T-cell infiltrates in the skin consisted of these same clones. Thus the manifestations observed in these patients correspond to features recorded in a large number of typical cases of Omenn syndrome.^{7,8,14}

The gene mutations causing Omenn syndrome remain elusive in nearly half of affected patients. Thus far, mutations in *RAG1/RAG2* or *Artemis* were shown to be associated with this SCID phenotype.^{8,14,18} Although null mutations in these genes cause complete abolition of recombination and a block in T- and B-cell development, mutations that hamper their enzymatic activity but permit residual function seem to allow for recombination in the few clones that are most likely at the heart of the multiorgan inflammatory process in Omenn syndrome.⁴⁻⁶

Both patients received allogeneic BMTs. Patient one received a haploidentical BMT that failed, followed by a successful matched unrelated donor BMT. Patient 2 received a matched related BMT from her older male sibling. Both recovered quickly, with full engraftments of T and B lymphocytes. The failure to gain weight resolved, but height remained delayed, prompting the consideration of a skeletal dysplasia. Skeletal survey, performed at 3 and 4 years in patients 1 and 2, respectively, clearly showed metaphysical dysplasia typical of CHH. If patients with CHH do not present at infancy with extreme obvious short-limb dwarfism, diagnosis can be delayed,³⁷ as happened in the cases presented here. Indeed, CHH demonstrates a high degree of pleiotropism among kindreds and even within families.³⁸ In Finland, where CHH is prevalent, patients present mainly with hair abnormalities, anemia, Hirschsprung disease, and a susceptibility to cancer.^{38,39} Growth retardation is variable, and subjects can reach adult height, ranging from 90 to 140 cm. Diagnosis of CHH at an early age might also be complicated by the absence of convincing metaphyseal changes early in life.³⁷ Dysplastic changes appear progressive and become apparent in childhood.^{2,29} Moreover, descriptions of CHH associated with SCID are rare. Indeed, in a large Finnish population with CHH, SCID was not identified, although a more recent report suggested early death in some of these patients,⁴⁰ confirming the original description by Mckusick et al²⁶ of Amish patients who died from overwhelming varicella infection. Actually, similar mutations have been recently identified in both populations.²⁴ Interestingly, the combination of short-limb dwarfism with Omenn syndrome has been previously observed.^{41,42} It is reasonable to assume that this case, like ours, is a unique presentation of CHH rather than representing a distinct syndrome.

Recently, it has been shown that CHH can be caused by mutations in the *MRP RNA* gene.^{20,21} The RNA encoded

by the gene is not translated into protein but is associated in a complex with the large MRP proteins. The complex was first described as having a role in the cleavage of mitochondrial primer RNA responsible for mitochondrial DNA replication, hence the name mitochondrial RNA processing, or RMRP.^{20,21} In yeast most of the RMRP is localized to the nucleolus, where it processes 5.8S ribosomal RNA, and it possibly affects translation of some proteins.⁴³ It has also been shown to have a role in regulation of the mitotic cell cycle and morphology of yeast.^{43,44} Indeed, mutations of RMRP in yeast lead to a prolonged and abnormal arrest in cell division as the result of an exit-from-mitosis defect caused by failure in degradation of cyclin B mRNA.⁴³⁻⁴⁵

The maturation of lymphocytes and immune responses are dependant on a high rate of cell division. If RMRP RNA has a similar function in human subjects, it might explain why aberrations in this gene might result in failure to develop normal mature lymphocytes, as seen in some cases of CHH.

The most common *RMRP RNA* gene mutation in the Finns and in the Amish is a homozygous 70 A-to-G nucleotide substitution.²⁴ Other patients, similar to ours, had a deleterious null mutation on one allele and a point mutation on the other allele. Point mutations identified in our patients occur at highly conserved positions of the RMRP RNA gene. In patient 1 we identified a new mutation, C4T, on the second allele. This mutation is at the second position of P1 hairpin structure that is conserved in *Xenopus* species, mice, rats, and human subjects.⁴⁶ The participation of this position in hairpin structure is supported by covariation in all species. Likewise, the new mutation identified in the second allele of patient 2 is in a 9-nucleotide stretch that is conserved in mouse, rat, bovine, and human RMRP RNA. These mutations are outside both the nuclear and mitochondrial localization signal regions and the known regions of RMRP RNA protein interactions.²³ However, the localizations of these mutations to a highly conserved hairpin structure and a conserved nucleotide stretch suggest an evolutionarily important function in either secondary structure or not-yet-identified protein RNA interactions.

In cases of combined heterozygotes, similar to our patients, RMRP RNA expression ranges from 35% to 50% of normal control expression.²⁴ Yet the clinical presentation of these patients appears heterogeneous, with no clear genotype-phenotype correlation. In fact, some of these cases are clinically indistinguishable from the common Finnish mutation, which results in no reduction in RMRP RNA expression.²² This shows a lack of correlation between RMRP RNA expression level and clinical features. Yet mutations that completely silence expression of *RMRP RNA* genes on both alleles have not been reported, presumably because a very low to complete absence of RMRP RNA expression is not compatible with life. It is possible that the immunodeficiency might be caused by genetic alterations, such as the point mutations found in our patients, which might affect interaction with proteins critical for the development of the immune system.

We have shown here that similar to *RAG* or *Artemis* mutations, the absence of normal RMRP RNA levels permits expansion of one or more T-cell clones, which presumably infiltrate the skin and lymphoid organs, leading to the phenotype of Omenn syndrome.

REFERENCES

- Omenn GS. Familial reticuloendotheliosis with eosinophilia. *N Engl J Med* 1965;273:427-32.
- Barth RF, Vergara GE, Khurana SK, Lonman JT. Rapidly fatal familial histiocytosis associated with eosinophilia and primary immunological deficiency. *Lancet* 1972;1:503-6.
- Wirt DP, Brooks EG, Vaidya S, Klimpel GR, Waldmann TA, Goldblum RM. Novel T-lymphocyte population in combined immunodeficiency with features of graft-versus-host disease. *N Engl J Med* 1989;321:370-4.
- Melamed I, Cohen A, Roifman CM. Expansion of CD3⁺ CD4⁺ CD8⁺ T cell population expressing high levels of IL-5 in Omenn's syndrome. *Clin Exp Immunol* 1994;95:14-21.
- Signorini S, Imberti L, Pirovano S, Villa A, Facchetti F, Ungari M, et al. Intrathymic restriction and peripheral expansion of the T-cell repertoire in Omenn syndrome. *Blood* 1999;94:3468-78.
- de Saint-Basile G, Le Deist F, De Villartay J-P, Cerf-Bensussan N, Jounet O, Brousse N, et al. Restricted heterogeneity of T lymphocytes in combined immunodeficiency with hypereosinophilia (Omenn's syndrome). *J Clin Invest* 1991;87:1352-9.
- Zhang J, Quintal L, Atkinson A, Williams B, Grunebaum E, Roifman CM. Novel Rag1 mutation in a case of severe combined immunodeficiency. *Pediatrics* 2005;116:e445-9.
- 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.
- Oettinger MA, Stanger B, Schatz DG, Glaser T, Call K, Housman D, et al. The recombination activating genes, RAG 1 and RAG 2, are on chromosome 11p in humans and chromosome 2p in mice. *Immunogenetics* 1992;35:97-101.
- Roman CA, Baltimore D. Genetic evidence that the RAG1 protein directly participates in V(D)J recombination through substrate recognition. *Proc Natl Acad Sci U S A* 1996;93:2333-8.
- Schatz DG, Oettinger MA, Baltimore D. The V(D)J recombination activating gene, RAG-1. *Cell* 1989;59:1035-48.
- Silver DP, Spanopoulou E, Mulligan RC, Baltimore D. Dispensable sequence motifs in the RAG-1 and RAG-2 genes for plasmid V(D)J recombination. *Proc Natl Acad Sci U S A* 1993;90:6100-4.
- Schwarz K, Gauss GH, Ludwig L, Pannicke U, Li Z, Lindner D, et al. RAG mutations in human B cell-negative SCID. *Science* 1996;274:97-9.
- 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.
- 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.
- Noordzij JG, Verkaik NS, van der Burg M, van Veelen LR, de Bruin-Versteeg S, Wiegant W, et al. Radiosensitive SCID patients with Artemis gene mutations show a complete B-cell differentiation arrest at the pre-B-cell receptor checkpoint in bone marrow. *Blood* 2003;101:1446-52.
- Ma Y, Pannicke U, Schwarz K, Lieber MR. Hairpin opening and overhand processing by an Artemis/DNA-dependent protein kinase complex in non homologous end joining and V(D)J recombination. *Cell* 2002;108:781-94.
- Ege M, Ma Y, Manfras B, Kalwak K, Lu H, Lieber MR, et al. Omenn syndrome due to Artemis mutations. *Blood* 2005;105:4179-86.
- Aleman K, Noordzij JG, de Groot R, van Dongen JJ, Hartwig NG. Reviewing Omenn syndrome. *Eur J Pediatr* 2001;160:718-25.
- Chang DD, Clayton DA. A mammalian mitochondrial RNA processing activity contains nucleus-encoded. *Science* 1987;235:1178-84.
- Clayton DA. A nuclear function for RNase MRP. *Proc Natl Acad Sci U S A* 1994;91:4615-7.
- Ridanpää M, van Eenennaam H, Pelin K, Chadwick R, Johnson C, Yuan B, et al. Mutations in the RNA component of RNase MRP cause a pleiotropic human disease cartilage-hair hypoplasia. *Cell* 2001;104:195-203.
- Ridanpää M, Sistonen P, Rockas S, Rimoin DL, Kakitie O, Kaitila I. Worldwide mutation spectrum in cartilage-hair hypoplasia: ancient founder origin of the major 70A→G mutation of the untranslated RMRP. *Eur J Hum Genet* 2002;10:439-47.
- Ridanpää M, Jain P, McKusick VA, Francomano CA, Kaitila I. The major mutation in the RMRP gene causing CHH among the Amish is the same as that found in most Finnish cases. *Am J Med Genet* 2003;121:81-3.
- Nakashima E, Mabuchi A, Kashimada K, Onishi T, Zhang J, Ohashi H, et al. RMRP mutations in Japanese patients with cartilage-hair hypoplasia. *Am J Med Genet* 2003;123:253-6.
- McKusick VA, Eldridge R, Hostetler JA, Ruangwitt U, Egeland JA. Dwarfism in the Amish. II. Cartilage-hair hypoplasia. *Bull John Hopkins Hosp* 1965;116:231-72.
- Makitie O, Kaitila I, Savilahti E. Susceptibility to infections and in vitro immune functions in cartilage-hair hypoplasia. *Eur J Pediatr* 1998;157:816-20.
- Makitie O, Kaitila I, Savilahti E. Deficiency of humoral immunity in cartilage-hair hypoplasia. *J Pediatr* 2000;137:487-92.
- Berthet F, Siegrist CA, Ozsahin H, Tuchschnid P, Eich G, Superti-Furga A, et al. Bone marrow transplantation in cartilage-hair hypoplasia: correction of the immunodeficiency but not of the chondrodysplasia. *Eur J Pediatr* 1996;155:286-90.
- Arpaia E, Shahar M, Dadi H, Cohen A, Roifman CM. Defective T cell signaling and CD8⁺ thymic selection in humans lacking Zap-70 kinase. *Cell* 1994;76:947-58.
- Sharfe N, Shahar M, Roifman CM. An Interleukin-2 receptor γ chain mutation with normal thymus morphology. *J Clin Invest* 1997;100:3036-43.
- Doherty PJ, Roifman CM, Pan S, Cymerman U, Ho SWK, Thompson E, et al. Expression of the human T cell receptor $\nu\beta$ repertoire. *Mol Immunol* 1991;28:607-12.
- Roifman CM, Hummel D, Martinex-Valdez H, Thorne P, Doherty PJ, Pan S, et al. Depletion of CD8⁺ cells in human thymic medulla results in selective immune deficiency. *J Exp Med* 1989;170:2177-82.
- Roifman CM. Studies of patients' thymic aid in the discovery and characterization of immunodeficiency in humans. In: Koretzky G, Monroe J, editors. *Immunological reviews*. Singapore: Blackwell Munksgaard; 2005. p. 143-55.
- Kuijpers TW, Ridanpää M, Peters M, de Boer I, Vossen JMJ, Pals ST, et al. Short-limbed dwarfism with bowing, combined immune deficiency, and late onset aplastic anaemia caused by novel mutations in RMRP gene. *J Med Genet* 2003;40:761-6.
- Gennery AR, Hodges E, Williams AP, Harris S, Villa A, Angus B, et al. Omenn's syndrome occurring in patients without mutations in recombination activating genes. *Clin Immunol* 2005;116:246-56.
- Bonafe L, Schmitt K, Eich G, Giedion A, Superti-Furga A. RMRP gene sequence analysis confirms a cartilage-hair hypoplasia variant with only skeletal manifestations and reveals a high density of single-nucleotide polymorphisms. *Clin Genet* 2002;61:146-51.
- Makitie O, Kaitila I. Cartilage-hair-hypoplasia—clinical manifestations in 108 Finnish patients. *Eur J Pediatr* 1993;152:211-7.
- Makitie O, Pukkala E, Teppo L, Kaitila I. Increased incidence of cancer patients with cartilage-hair hypoplasia. *J Pediatr* 1999;134:315-8.
- Makitie O, Pukkala E, Kaitila I. Increased mortality in cartilage-hair hypoplasia. *Arch Dis Child* 2001;84:65-7.
- Gatti RA, Platt N, Pomerance HH, Hong R, Langer LO, Kay HE, et al. Hereditary lymphopenic agammaglobulinemia associated with a distinctive form of short-limbed dwarfism and ectoderm dysplasia. *J Pediatr* 1969;75:675-84.
- Schofer O, Blaha I, Mannhardt W, Yepp F, Stallmach T, Spranger J. Omenn phenotype with short-limbed dwarfism. *J Pediatr* 1991;118:86-9.
- Li X, Zaman S, Langdon Y, Zengel JM, Lindahl L. Identification of a functional core in the RNA component of RNase MRP of budding yeasts. *Nucleic Acids Res* 2004;32:3703-11.
- Gill T, Cai T, Aulds J, Wierzbicki S, Schmitt ME. RNase MRP cleaves the CLB2 mRNA to promote cell cycle progression: novel method of mRNA degradation. *Mol Cell Biol* 2004;24:945-53.
- Cai T, Aulds J, Gill T, Cerio M, Schmitt ME. The *Saccharomyces cerevisiae* RNase mitochondrial RNA processing is critical for cell cycle progression at the end of mitosis. *Genetics* 2002;161:1029-42.
- Schmitt ME, Bennett JL, Davaghi DJ, Clayton DA. Secondary structure of RNase MRP RNA as predicted by phylogenetic comparison. *FASEB J* 1993;7:208-13.