case report

Severe combined immunodeficiency caused by a new homozygous RAG1 mutation with progressive encephalopathy

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We describe an unusual case of severe combined immunodeficiency (SCID) with neutropenia and central nervous system (CNS) manifestations in which a novel RAG1 mutation was identified. A 15-month-old boy presented with failure to thrive, neutropenia and recurrent infections. He was diagnosed with T-B-NK+ SCID. He subsequently developed right partial seizures with ipsilateral hemiparesis and became comatose. Magnetic resonance imaging (MRI) of the brain revealed an inflammatory lesion in the left thalamus which later progressed to diffuse meningo-encephalitis on serial imaging. No CNS infection was documented. Genetic work-up in the child revealed a novel homozygous deleterious mutation in the RAG1 gene (c:2881T>C; p:I794T), for which both parents were heterozygous. He underwent a haplo-identical bone marrow transplant without conditioning and died on day +35 with no improvement in his neurological status. The features of neutropenia and progressive encephalopathy could be linked to the novel genetic defect but more data is required to establish this conclusively.

Severe combined immunodeficiency (SCID) represents a heterogeneous group of disorders characterised by impairment in both cellular and humoral immunity. SCID arises from mutations of genes critical to the development of the adaptive immune system. The defining characteristic is a profound depletion of circulating lymphocytes resulting in markedly increased susceptibility to severe infections from early infancy. The only form of curative therapy for this potentially fatal disorder is hematopoietic stem cell transplantation. Recombinase activating genes 1 and 2 (RAG1 and RAG2) play a vital role in the process of re-arrangement of the variable (V), diversity (D) and joining (J) segments during the development of the T cell and immunoglobulin receptors, a process crucial for antigenic diversity.[1] The association between B cell negative SCID and RAG1 and RAG2 genes was first described by Klaus et al. in 1996.[2] Since then numerous mutations at

RAG1 or RAG2 locus on chromosome 11p23 have been described, resulting in a diverse clinical spectrum.[3] These mutations are broadly classified into RAG deficient SCID (RAGD) with no V (D) J recombination (<1% recombination activity of wild type), and RAGD with residual V (D) J recombination (>1% recombination activity of wild type).[4] We report an unusual case of T-B-NK+ SCID with neutropenia and progressive encephalopathy in which a novel homozygous missense RAG1 mutation was identified.

CASE REPORT

We report the case of an 11-month-old boy of Indian descent, second born of a consanguineous marriage who was delivered at term after an uneventful pregnancy. He presented with complaints of failure to gain weight, recurrent respiratory tract infections and loose

stools since the age of 5 months. Developmentally, the child exhibited features of mild motor and mental impairment. He had no vision, hearing or motor deficits. He had received multiple courses of oral (amoxicillin–clavulonate and ofloxacin) and intravenous antibiotics (ceftriaxone) in the past but his symptoms persisted and he was referred to our centre for further evaluation. At presentation the child was febrile and tachypneic with mild chest retractions. Respiratory system examination revealed coarse bilateral crepitations. There was no organomegaly, lymphadenopathy, oral thrush, ear discharge or skin rash. The rest of the systemic examination was unremarkable. Complete blood count done on admission showed a haemoglobin of 9.9 g/dl, total leukocyte count (TLC) of 5300/μl with an absolute neutrophil count (ANC) of 159/μl, an absolute lymphocyte count of 2800/μl and platelet count of 265000/μl. Peripheral smear showed microcytic hypochromic anemia with no evidence of hemolysis. To reduce cost of investigation in this admission, a bone marrow was not performed. Tests for hepatic and renal function were normal. Chest X-ray showed evidence of bilateral diffuse pneumonitis with a normal/small thymic shadow. Anti-mycobacterial therapy (AMT) comprising isoniazid, rifampicin, ethambutol and pyrizinamide was started presumptively in view of maternal history of tuberculosis which was adequately treated 9 months back. His blood culture and bronchio-alveolar lavage were negative for any bacterial or fungal pathogen. On immunological evaluation he was found to have hypogammaglobulinemia, T and B cell lymphopenia (CD4+, CD8+ and CD19+) and normal CD56+ cells. Detailed investiga-

Table I. Summary of immunological evaluation.

	Patient	Normal range
Immunoglobulins (mg/dl)		
Ig A	1	4–90
Ig M	17	25–150
Ig G	89	200–1000
Ig E	3.6	<35
Peripheral blood lymphocyte subset		
CD 3	576/μl	1460–5440/μl
CD 4	320/μl	1020–3600/μl
CD 8	134/μl	570–2230/μl
CD 19	6/μl	430–3300/μl
CD 56	369/μl	80–340/μl

tions are summarised in Table I. He was diagnosed as a case of T-B-NK+ severe combined immunodeficiency (SCID) with neutropenia, and advised regarding a hematopoetic stem cell transplant. His elder sibling, a six-year-old female was only a 3/6 match at HLA loci, and a search for an unrelated donor unit was initiated but his parents were unwilling to have an unrelated donor transplant. He was discharged on request on AMT, antifungal (fluconazole), co-trimoxazole for Pneumocystis jirovici prophylaxis and intravenous immunoglobulin at three-week intervals.

The child was readmitted to the hospital 4 months later on account of fever, diarrhoea and right henicovulsions. He was in a state of shock and the sensorium was obtunded. Emergency measures were instituted with parenteral fluids, antibiotics and anticonvulsants. His condition stabilised, the sensorium remained obtunded and a right hemiplegia was detected. Investigations revealed a haemoglobin of 9.2 g/dl, TLC of 1400/μl and platelet count of 14,000/μl and an absolute neutrophil count (ANC) of 336/μl. Serum electrolytes, hepatic and renal functions were in the normal. MRI of the brain revealed a $2 \times 1.2 \times 2$ cm area in the left thalamus, showing bright signal intensity on T-2 weighted images and low signal intensity on T-1 weighted images (Figure 1A). The possibility of an infarction or infective pathology was considered. A lumbar tap yielded cerebrospinal fluid (CSF) under normal pressure. Biochemistry, cytology and staining for bacteria and fungi were negative. A detailed work-up for an infective etiology was conducted. Cultures and PCR studies were done in blood and found to be negative for all common bacterial, fungal and viral pathogens which included toxoplasma, mycobacterium tuberculosis, mycoplasma pneumonia, cytomegalovirus, Ebstein–Barr virus, parvovirus, rubella, herpes-simplex virus, JC virus, human immunodeficiency virus, herpes-simplex virus, Japanese encephalitis and adenovirus. We could not test for poliovirus, enterovirus and measles. Testing for antibodies against NMDA (N-methyl D-aspartate) receptor was also negative in blood and CSF. The details of investigations conducted are summarised in Table II. His chest X-ray showed persistent bilateral nodular infiltrates and AMT was continued. He was empirically started on treatment with broad spectrum antibiotics (Meropenem/Amikacin/Teicoplanin), antifungal (IV caspofungin), antiviral (IV acyclovir), antiprotozoal (sulfadoxine–pyrimethamine) drugs, and anti-convulsants (sodium valproate, phenobarbitone and levetiracetam). IVIG infusions were given to boost immunity. The condition of the child remained critical. Follow-up MRI brain scans showed extensive dis-
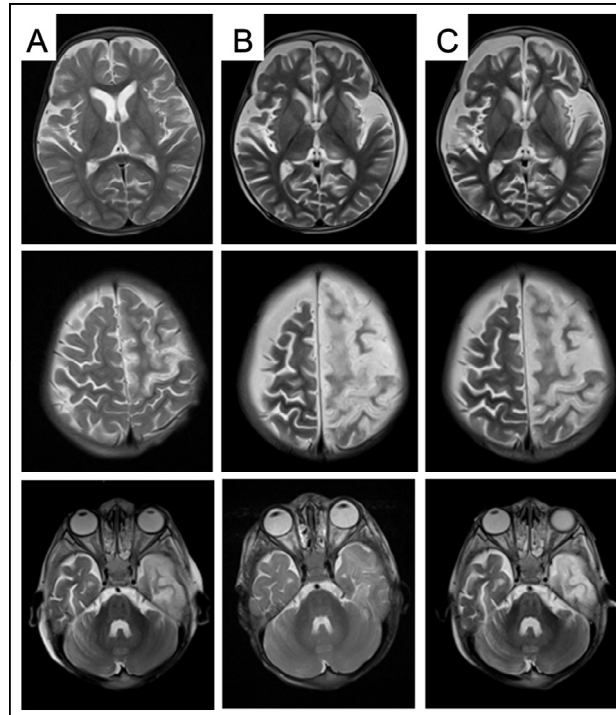
Figure 1. The MRI done at admission (A) reveals hyper-intensities predominantly in the left temporal and frontal lobes with hyper-intense foci seen in the left thalamus and subtle hyper-intensities in the brainstem. The scan obtained 3 weeks later (B) shows rapid progression of the disease with increased areas of signal abnormality in both cerebral hemispheres as well as the brainstem. The brain shows early atrophic changes. On the last scan (C) the atrophic/gliotic changes have progressed further with areas of T1 hyper-intensity. No diffusion restriction or enhancement were noted at any time.

tribution affecting left thalamus, medial occipital region, left insular cortex and cingulate gyrus. Increased signal intensities were noted in the right cingulate gyrus and temporo-parietal areas, affecting the cortex and white matter (Figure 1B). MR arteriography and venography excluded an arteritis or occlusive cerebrovascular disease. Electro-encephalography (EEG) revealed bilateral periodic lateralised epileptiform discharges (bi-PLEDs) and later EEGs showed a burst suppression pattern. In view of his altered sensorium and uncontrolled seizures, emergency management of refractory/super-refractory status epilepticus with midazolam continuous infusion, increased dosing of ongoing anti-convulsants was carried out. The child was intubated and mechanical ventilation instituted. At this juncture, the parents insisted on definitive therapy in the form of stem cell transplant, and they were counselled appropriately regarding the risks and prognosis. However, after detailed discussion with the family, a decision was taken to proceed with a haploidentical stem cell transplant with the mother as donor. Pre-transplant chimerism analysis of the patient did not show any evidence of maternal microengraftment. Unmanipulated bone marrow graft was infused with a CD34 cell dose of 5.5 million/kg of re-

cipient weight. No conditioning was given, and posttransplant immune-suppression comprising cyclophosphamide (50 mg/kg) on day +3 and day +4, tacrolimus (1 mg/kg once daily) and mycophenolate mofetil (15 mg/kg thrice daily) from day +5, was given. Tacrolimus levels were monitored and doses modified to maintain trough levels between 5 and 10 ng/ml. The patient continued to receive prophylactic antibiotics, anti-fungal, antiviral and anti-tubercular drugs as described before. FISH for XX and XY chromosomes to look for engraftment on day +19 showed 2% maternal cells, but was subsequently negative for donor cells on day +29, and day +35. Post-transplant, he remained on mechanical ventilation and seizures persisted. The patient also had persistent dyselectrolytemia (hypernatremia and hypokalemia), hypoalbuminemia, and hypertension which were medically managed. Irradiated blood products were given as required. Neuro-imaging was repeated in view of seizures which showed extensive encephalomalcia in both cerebral hemispheres (Figure 1C). He remained obtunded with GCS of 3/15.

The patient developed multi drug resistant *Chryseobacterium indologenes* sepsis with multi-organ failure. Antibiotics were changed as per blood culture

Table II. Details of investigations at the onset of neurological symptoms.

Blood	
Complete blood count	Hb-9.2 g/dl, TLC-1400/µl, platelet count – 14,000/µl, ANC-336/µl
Culture	Negative
CMV PCR (quantitative)	Negative
EBV PCR	Negative
Toxoplasma PCR	Negative
CSF	
Cell count and differential	2 lymphocytes
Protein/sugar(mg/dl)	16/84
Culture	Negative
EBV PCR	Negative
CMV PCR	Negative
HSV PCR	Negative
Toxoplasma PCR	Negative
JC virus PCR	Negative
Adenovirus PCR	Negative

sensitivity reports. He died on day +35 post-transplant with rejection of donor cells and no improvement in the neurological status. A brain biopsy was planned, but was refused by the family.

Blood samples of the patient and both parents were sent to France for precise identification of the genetic defect. Molecular sequencing revealed a novel homozygous mutation in the RAG1 gene (c:2381 T>C; p:I794T) in the child (Figure 2) for which both parents were heterozygous. Sequencing of RAG2 locus and Artemis genes did not reveal any abnormality.

DISCUSSION

Mutations in RAG1 and RAG2 genes account for 50% of cases of T-B-NK+ SCID.[5] The spectrum of disorders resulting from RAG1 and RAG2 mutations varies from classical SCID and classical SCID with materno-fetal transfusion (with no V (D) J activity) to partial deficiency resulting in Omenn's syndrome and granulomatous inflammation.[4] In our case, we could not test for RAG protein expression and functional assay for lymphocyte proliferation as these tests are not available in India. This disorder is mostly associated with a T-B-NK+ phenotype but other variants are also described.[6] We identified a novel mutation in the RAG1 gene for which the child was homozygous and both parents were found to be heterozygous. He had an unusual clinical phenotype which we hypothesised to be related to the unique genetic defect.

This patient had initially presented with isolated neutropenia but later on he developed depression of all cell lines. Classically severe neutropenia has been described in conjunction with reticular dysgenesis.[7] Niehues et al. described neutropenia in a case of SCID with materno-fetal transfusion which was responsive to G-CSF therapy.[8] Radiosensitive SCID variants with defects in non-homologous end joining
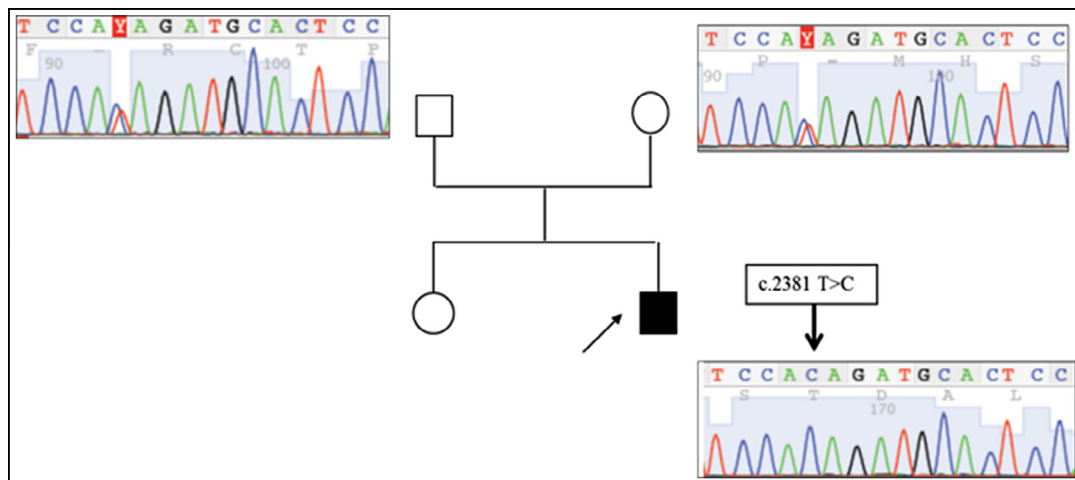

Figure 2. Pedigree chart showing a novel homozygous mutation identified as RAG1: c.2381 T>C; p. Ile794Thr in the child.

have features akin to Nijmegen breakage syndrome[9] and typically present with pancytopenia. These include ligase-IV syndrome,[10] and hypomorphic mutations in Artemis.[11] In our patient, the neutropenia at presentation could be ascribed to the multiple antibiotics he had received previously – all of which are known to cause agranulocytosis. However, we speculate that it could be linked to his genetic defect. In RAGD, neutropenia has not been described so far and we believe that this is peculiar to the new mutation identified in our patient.

Central nervous system (CNS) involvement in SCID is a rare occurrence and difficult to diagnose as signs and symptoms may be subtle and a high index of suspicion is necessary. It is usually secondary to infections or neurotoxic medications but in certain variants of SCID, primary CNS pathology has also been described. Neurological manifestations are most commonly seen in patients with adenosine deaminase (ADA) deficiency and include motor dysfunction, developmental delay, cortical blindness, sensorineural deafness and behavioural abnormalities.[12,13] In ADA-deficient SCID, the CNS insult is thought to result from toxic accumulation of adenosine in the brain especially in areas rich in adenosine receptors such as the basal ganglia and thalamus. These abnormalities may persist or can occur post-transplant as well.[14] Recent data has thrown light on association of FOXN1 mutations in NUDE/fetal SCID with multiple site neural tube defects, thymic aplasia as well as alterations of corpus callosum and reduction in cerebellar Purkinje cells.[15–17] Interestingly in our patient too, the lesion was first noted in the thalamus. Other forms of SCID with CNS damage are DNA ligase IV deficiency,[10] and spastic diplegia, characteristically seen in purine nucleoside phosphorylase deficiency.[18] CNS infections in SCID are infrequently described in literature. Waruiru et al. found an incidence of 10% in their cohort of 79 patients of SCID.[19] They described eight patients of SCID (including three with RAGD and two with T-B-NK+ SCID of undetermined origin) with viral infections in CNS confirmed by PCR-based techniques. CSF cell count and biochemistry was abnormal in four cases. The CSF analysis in our case was absolutely normal. The aetiopathogenesis of the acute and progressive CNS deterioration in our patient remains undecided. The primary manifestation of immunodeficiencies is undue susceptibility to infection. Progressive neuro-degeneration in primary immunodeficiency has been demonstrated in experimental animals and humans. Ziegner et al. reported 14 patients with diverse primary immunodeficiencies

of whom one child had SCID and developed a progressive neurodegenerative process. These patients were on chronic IVIG therapy. The course of the neurological illness was chronic, no infectious agent was isolated; and neuro-imaging revealed cerebral atrophy with ventricular dilation.[20] In our patient, an infectious etiology seems most plausible. The neurological illness occurred in the background of diarrhoea and pneumonia. Recurrent convulsions, hemiplegia and altered sensorium occurring acutely indicated an infection, auto-immune process or a vascular etiology. However, involvement of the limbic structures such as the insular cortex, cingulate gyrus, and temporal areas on MR imaging combined with bi-PLEDs would suggest an infective etiology such as herpes simplex virus or CMV infection. Moreover, confirmatory evidence in the form of virus isolation or was not forthcoming. A brain biopsy was not agreed to. Neurological manifestations have not been described so far in RAG1 mutations. Whether the new genetic defect identified in this patient may have a predilection for CNS involvement by mechanisms yet to be discovered, or whether he had unidentified infective encephalitis, cannot be ascertained. However, due to the consanguinity background, there is also a possibility that two independent disorders may co-segregate. The likelihood of a second disorder like mitochondrial or genetic neurological disorder is possible but we could not test for them.

The pioneers in the field of haploidentical stem cell transplants for SCID, Reisner et al., successfully achieved stable immune reconstitution in SCID patients with T-cell depleted HLA-haploidentical parental grafts.[21] Since then several other groups have reported success rates varying from 54% to 77% for haplo-transplants.[22,23] Factors found to be predictive of a poor outcome are an older age at transplant (>5 months), B-SCID, functional NK cells, infections, and graft versus host disease.[22,23] Most authors have shown higher success rates with the use of a cytoreductive conditioning regimen and T cell depletion of the graft. However, T cell depletion is best avoided in the presence of active infection. Children with viral CNS infections have a dismal course even if transplant is done at an appropriate time.[15] Taking into consideration the unfavourable circumstances in our child, we opted for a T-cell replete graft, and followed a mini-haplo transplant protocol which has been described in 217 patients with advanced haematological malignancies.[24] We omitted the conditioning regimen in this case as pre-existing organ damage was extensive and fludarabine is known to be neurotoxic. Cyclophosphamide was given post-transplant on day

case report

+3 and day +4. There is compelling evidence derived from both murine and human models which suggests that cyclophosphamide administered at a critical time point post-transplant selectively ablates activated allo-reactive T-lymphocytes, and prevents graft versus host disease.[25] The rationale for choosing the mother as the donor was derived from studies which have shown that transplacental passage of maternal cells during pregnancy establishes long term maternal micro-chimerism in the child, which facilitates engraftment by inducing immune tolerance to the graft.[26]

Factors contributing to non-engraftment in our patient were the absence of maternal cells in the child,

delay in transplant, NK+ phenotype, and a lack of conditioning regimen.

The distinctive features in this case were the presence of neutropenia and a progressive encephalopathy. We suspect that these features could be linked to the novel genetic defect but more data is required to establish this conclusively.

CONFLICT OF INTEREST

All authors have no conflict of interest to declare and have nothing to disclose.

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