A novel compound heterozygous HAX1 mutation in a Chinese patient with severe congenital neutropenia and chronic myelomonocytic leukemia transformation but without neurodevelopmental abnormalities

Severe congenital neutropenia (SCN) is a rare disease, comprising a heterogeneous group of inherited disorders. Clinical features include reduced absolute neutrophil counts from birth, increased susceptibility to recurrent and life-threatening infections, and a pre-leukemic predisposition.[1]

SCN exhibits different genetic modes of inheritance, including autosomal dominant, autosomal recessive, X-linked and sporadic forms. Specific genetic mutations have been described for the different types of SCN.[2] Homozygous mutations in HAX1 are the pathogenic mutations in autosomal recessive SCN.[3] However, compound heterozygous HAX1 mutations have also been identified in these patients.[4-6] A correlation between genotype and phenotype was observed in these patients, suggesting that mutations affecting transcript variant 1 of HAX1 were associated with CN alone, whereas mutations affecting both transcripts caused CN and neurological symptoms.[6-8] This finding implies isoform b of the HAX1 protein is critical for neuronal function, which has also been confirmed in an animal experiment.[9] The present study describes the first Chinese autosomal recessive SCN patient with chronic myelomonocytic leukemia (CMML) transformation, who carries a novel compound heterozygous mutation in the *HAX1* gene that affects both transcripts without neurodevelopmental abnormalities.

This 20-year old patient was referred to us in April 2009 for persistent neutropenia. Repeated full blood counts showed leukopenia and neutropenia without monocytosis. Bone marrow analysis revealed a maturation arrest at the promyelocyte/myelocyte stage with few mature neutrophils. No cytogenetic abnormalities were found and cranial magnetic resonance imaging was normal.

Past medical history revealed recurrent and refractory oral ulcers, chronic gingivitis and frequent bouts of fever of no obvious origin from soon after birth. He had never received G-CSF. By 17 years of age, the patient had splenomegaly, hepatomegaly, cervical lymphadenopathy and thickening of the walls of the ascending colon with a dilated lumen. Splenectomy and biopsies of the affected tissue were performed and showed non-specific inflammatory responses to an acute or chronic inflammatory stimulus.

The patient performed well at school and is now a skilled craftsman. No cognitive problems or neurodevelopmental delay were observed, and there was no relevant family history.

A diagnosis of SCN was made but no treatment was given as the patient felt well. During a routine follow-up visit in February 2010, CMML was diagnosed. This was confirmed by a bone marrow smear showing dysplastic erythrocytes and megakaryocytes, with persistent peripheral blood monocytosis (>1×10^9/L). No cytogenetic abnormalities, including Bcr/abl, PDGFRA or PDGFRB, were detected.[10]

To clarify the pathogenic mutation in this patient, genomic DNA was extracted from bone marrow samples before and after developing CMML. Sequencing analysis of the candidate genes proved that the patient had a novel compound heterozygous HAX1 mutation consist-

Table 1. Demographic data and clinical information of patients with compound heterozygous HAX1 mutations.

Patient n.	Nationality	Gender	At diagnosis Age	WBC (/μL)	ANC (/μL)	Mutations at Mutation location	Nucleotide level	Protein level	G-CSF R	Therapy	Outcome	Neurological symptoms	Ref.
1	Japanese	F	12 m	4170	376	Exon 2b/3	c.256C>T /c.376-434del59bp	p.Arg86X /p.Arg126fsX128	ND	URD-HSCT (aged 5 yrs)	Alive, aged 6 years. After HSCT, no complication and no obvious improvement in cognitive function or behavior occurred.	Moderate neurodevelopmental delay, no epilepsy.	6
2	Japanese	M	6 m	6710	0	Exon 2b/3	c.256C>T /c.376-434del59bp	p.Arg86X /p.Arg126fsX128	ND	G-CSF	Alive, aged 5 years.	Mild neurodevelopmental delay, no epilepsy.	6
3	Swedish	M	2 m	ND	100-500	Exon 2a/5	c.91delG /c.568 C>T	p.Glu31LysfsX54 /p.Gln190X	ND	G-CSF	Alive, aged 15 years.	No	4
4	Italian	M	7 m	ND	100	Exon 3/3	c.389T>G /c.430-1insG	p.Leu130Arg /p.Val144GlyfsX5	ND	G-CSF	Alive, aged 4 years.	Neurodevelopmental delay combined with pathological EEG, no epilepsy.	5
5	Chinese	M	20 yr	2080	320	Exon 3/5	c.430-1insG /c.655-9del5bp	p.Val144GlyfsX5 /p.Pro219TrpfsX13	WT	No	Alive, aged 22 years. Progressed to CMML at the age of 21 years.	No	This report

ND: not determined; URD-HSCT: unrelated donor hematopoietic stem cell transplantation.

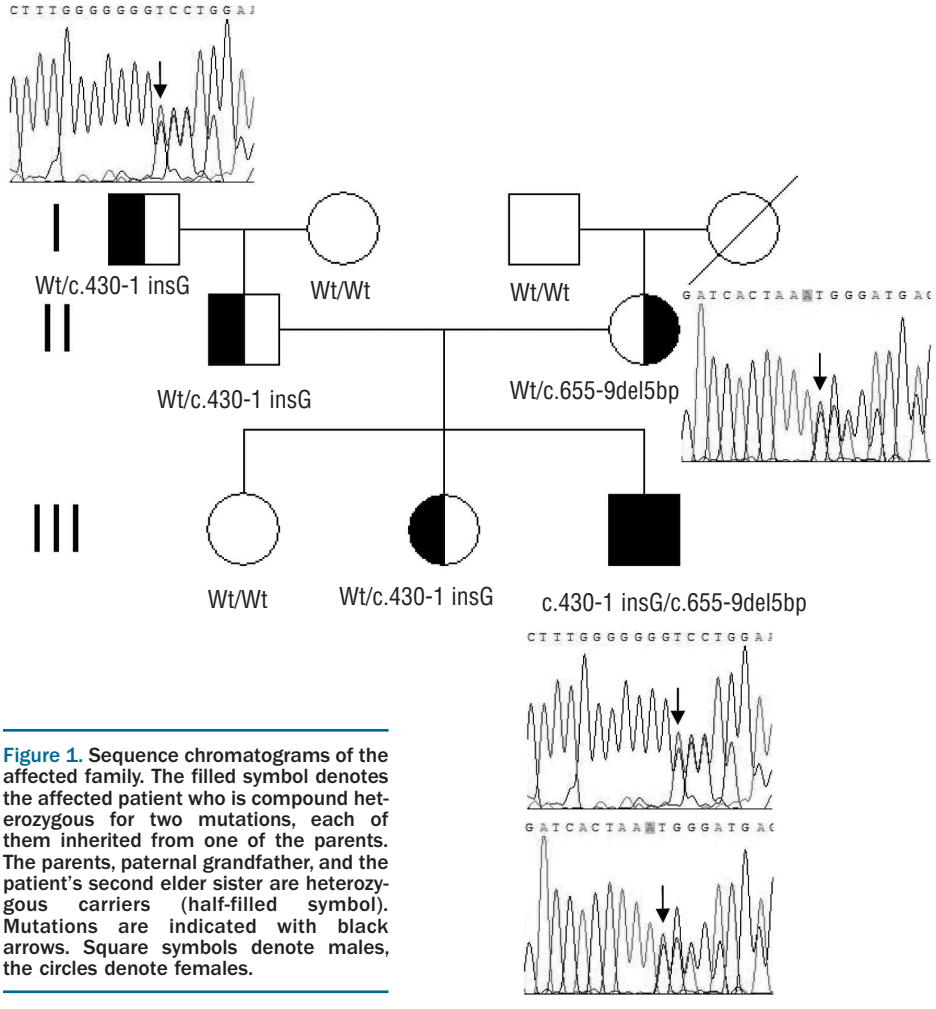

Figure 1. Sequence chromatograms of the affected family. The filled symbol denotes the affected patient who is compound heterozygous for two mutations, each of them inherited from one of the parents. The parents, paternal grandfather, and the patient's second elder sister are heterozygous carriers (half-filled symbol). Mutations are indicated with black arrows. Square symbols denote males, the circles denote females.

ing of two frame-shift mutations which resulted in premature stop codons. One was c.430-1insG (exon 3) which has been described previously.[5,7] The other mutation, c.655-9del5bp (exon 5), causes p.Pro219TrpfsX13; to the best of our knowledge this is a novel mutation.

To define the inheritance pattern of this novel mutation, the *HAX1* gene was sequenced in genomic DNA from peripheral blood of all other family members. The paternal grandfather, father and second elder sister of the proband were heterozygous carriers of the c.430-1insG mutation; the proband's mother was a heterozygous carrier of the c.655-9del5bp mutation, and all carriers had a normal phenotype. The HAX1 protein was detected in the carriers of both types of HAX1 mutation by Western blotting, but the patient had a deficiency in HAX1 protein levels. Figure 1 shows the pedigree of the autosomal recessive inheritance pattern.

The co-relationship between genotype and phenotype in HAX1 mutations has been revealed by several research groups in SCN patients.[6-8] This correlates with compound heterozygous HAX1 mutations reported previously (Table 1).[4-6] Such a correlation was not the case in our current patient as there was no neurodevelopmental abnormality. A SCN patient with neurological symptoms due to homozygous HAX1 mutation of c.430-1insG has been described.[7] Therefore, we speculate that the p.Pro219TrpfsX13 mutation produced by c.655-9del5bp could replace the role of the isoform b of HAX1 in neuronal function. This protein probably could not be detected in our patient by a monoclonal antibody to the HAX-1 amino acid 10-148 sequence due to the altered tertiary structure.

To understand more about SCN patients with compound heterozygous HAX1 mutations, we reviewed the patients reported in previous studies and summarized their characteristics in Table 1. They all presented typical clinical phenotypes of CN, as summarized.[1] They could also show neurological manifestations but no epilepsy was recorded, while for SCN patients with homozygous HAX1 mutations common to transcript variants 1 and 2, all of these showed epilepsy except the mutation of c. 568 C>T.[3,5-8,11]

Although CMML may have developed in our patient independently, an increased risk of malignant transformation in patients with SCN has been well documented and our patient had wild genotypes of G-CSFR, KRAS, NRAS and no other cytogenetic abnormalities.[12] Whole genome sequencing of this patient is being performed to identify the mutated genes that contributed to the malignant transformation.

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POEMS syndrome with severe neurological damage clinically recovered with lenalidomide

POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal component, and skin changes) is a paraneoplastic disorder associated with an underlying plasma cell dyscrasia. Associated features to this syndrome are sclerotic bone lesions, elevated levels of vascular endothelial growth factor (VEGF), Castleman's disease, endocrinopathies, papilledema, peripheral edema, effusions, ascites, thrombocytosis and fatigue.[1] The pathogenesis of the POEMS syndrome is not well understood, but it is likely that proangiogenic[2] (such as VEGF) and proinflammatory cytokines[3] (such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1 and interleukin (IL)-6) play an important role in the pathogenesis of this disease.

Therapy for POEMS syndrome has not been standardized. So far, many different strategies have been used, including radiotherapy, chelating agents and corticoids, immunoglobulins, plasmapheresis, and high doses of chemotherapy followed by peripheral blood stem cell transplant (SCT).[4] Monoclonal antibodies, like rituximab (anti-CD20) or bevacizumab (anti-VEGF), have also been used. Recently, immunomodulatory drugs (IMiDs), such as thalidomide or lenalidomide, have emerged as therapeutic options in these patients because they are powerful drugs against malignant plasma cells which reduce the production of proinflammatory and proangiogenic cytokines. Both drugs have shown efficacy in improving the clinical condition of patients with POEMS syndrome.[5-7] Lenalidomide has the additional advantage of being associated with a much lower risk of peripheral neuropathy than thalidomide. Similarly, concerns about exacerbating neuropathy also arise when other new therapeutic agents, such as bortezomib, are taken into consideration.

We present the clinical outcome of 10 patients with POEMS syndrome who were treated at different centers in Spain with lenalidomide as salvage therapy from 2007 to 2010. This restrospective study was approved by the MD Anderson Cancer Center Madrid Scientific Committee (Institutional Review Board). Age at entry ranged from 39 to 63 years and patients' condition fulfilled published diagnostic criteria.[1] Main clinical features of POEMS syndrome are described in Table 1. All patients had motor-sensory polyneuropathy, predominantly affecting the lower limbs, monoclonal plasmaproliferative disorder and sclerotic bone lesions. Of note, neurological symptoms led to a restrictive ventilatory alteration in 2 cases (ns. 4 and 5), and confined patients to a wheelchair or to bed in 7 cases (ns. 3, 4, 5, 6, 7, 8 and 10).

All patients had received previous treatments to lenalidomide therapy (Table 2), including 4 cases who had received chemotherapy (melphalan-containing regimens, cyclophosphamide or polychemotherapy treatment), one who was treated with rituximab, and 6 who had received immunoglobulin treatment. Local radiation therapy was administered in 2 patients for sclerotic