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A RARE CASE OF EMBERGER SYNDROME CAUSED BY A DE NOVO MUTATION IN THE GATA2 GENE

S. Michelini, M. Cardone, M. Haag O Agga, A. Bruson, P.E. Maltese, A. Bonizzato, M. Bertelli

Department of Vascular Rehabilitation (SM,MC,MHOA), San Giovanni Battista Hospital ACISMOM, Rome; MAGI Non-Profit Human Medical Genetics Institute (AB,PEM,AB,MB), Rovereto (TN), Italy

ABSTRACT

Emberger syndrome, or primary lymphedema with myelodysplasia, is a severe rare disease characterized by early primary lymphedema and blood anomalies including acute childhood leukemia. The syndrome is associated with heterozygous mutations in the GATA2 *gene. We report on a 13-year-old boy who developed lymphedema of the right lower limb at age 6 years which was accompanied by severe panleukopenia and repeated episodes of erysipelas. The suspicion of Emberger syndrome was confirmed by detection of a new germinal line* GATA2 *mutation c.414_417del, p.Ser139Cysfs*78. Clinical treatment included a bone marrow transplant from the father. This case is one of a very limited number of Emberger syndrome cases documented in the literature, and genetic testing proved fundamental for definition of the condition and its association with a* de novo *mutation in the* GATA2 *which is reported here for the first time.*

Keywords: Emberger syndrome, primary lymphedema, *GATA2*, *de novo* mutation

Emberger syndrome (OMIM: 614038) has been observed in a very limited number of families and is classified as a very rare disease (prevalence <1/1,000,000, Orphanet). Described for the first time by Emberger in a family with three affected members belonging to different generations (1), it manifests with sensorineural deafness, early onset primary lymphedema of the lower limbs, and blood anomalies (acute myeloblastic leukemia, cytopenia). Lymphedema affects one or both legs and sometimes the genitalia. Other features may include severe diffuse warts, hypotelorism, epicanthus, long tapered fingers, recurrent cellulitis of the affected limb, and mutism. Pancytopenia or myelodysplasia are often associated. Since Emberger's report, very few new cases with similar features followed (2,3), and only in 2011 was the gene causing this rare disease identified at St. George Hospital, London. In an article published in Nature Genetics, Ostergaard et al (4) used exome sequencing in three unrelated subjects to identify *GATA2* (OMIM: 137295) as the gene for which mutations cause Emberger syndrome. The gene *GATA2* (GATA Binding Protein 2) (NCBI SeqRef: NM_032638.4; NP_116027.2) codes for a protein of the transcription factor family, named after the consensus nucleotide sequence for binding to promoter regions of target genes. It contains two Zinc-finger domains for binding to DNA and is expressed in both hemopoietic progenitor cells and embryonic non-hemopoietic stem cells and therefore plays an essential role in regulating transcription of genes involved in the development and proliferation of hemopoietic cells.

Subsequently, five other independent

affected subjects were analyzed by direct sequencing of the *GATA2* gene and a total of eight different heterozygous mutations were identified, six of them leading to the formation of a truncated protein. The other variants, p.R361L and p.C373R, lead to alteration of conserved residues in the DNA binding domain (4). Transmission is autosomal dominant.

MATERIALS AND METHODS

All clinical assessments were conducted at San Giovanni Battista Hospital ACISMOM (Rome, Italy). Lymphoscintigraphy, color Doppler echography, and magnetic resonance lymphangiography were performed in the Department of Vascular Rehabilitation where Emberger syndrome was first suspected. Testing for mutations in the coding region of the *GATA2* gene (Accession Number NM_032638.4) was performed at MAGI Non-Profit Human Medical Genetics Institute (Rovereto, Italy). DNA of the proband and his parents was extracted from whole blood (Blood DNA Kit E.Z.N.A.; Omega Bio-Tek Inc., Norcross, GA, USA) and analyzed by PCR and direct sequencing of the five coding exons and the corresponding intron-exon junctions with a Beckman Coulter CEQ 8000 sequencer (Beckmann Coulter, Milano, Italy). Amplification primers and experimental conditions are available on request.

To identify mutations previously reported as pathogenic, the Human Gene Mutation Database (HGMD) professional (Release 2014.1) (http://www.biobase-international.com/product/hgmd) was used. In order to exclude polymorphisms, we also searched the public database of single nucleotide variants (dbSNP, www.ncbi.nlm.nih.gov/SNP/) and the Exome Variant Server (EVS) database (http://evs.gs.washington.edu/EVS/).

The study followed institutional guidelines and the Declaration of Helsinki and all subjects signed an informed consent to make their clinical and genetic data available for publication of this case report.

Fig. 1. Patient age 6 years showing lymphedema of the right lower limb and genitals.

CASE SUMMARY

The proband was a 13-year-old boy who developed lymphedema of the right lower limb at 6 years of age (*Fig.1*).

Color Doppler ultrasonography of the lower limbs was negative for venous pathologies. The child had a family history of thrombophilia: the mother had heterozygous mutations in *MTHFR* and Factor V and homozygous mutations in *PAI-2*. At the initial presentation, blood chemistry showed panleukopenia with peaks of WBC 2.66 10^3/µl (performed at 10 years of age during hospital admission for major anterior nosebleed) (*Table 1*).

TABLE 1
Routine Blood Chemistry Tests

Blood Chemistry Tests	2012	2013	2014 (before BM transplantation)	2015 (after BM transplantation)
WBC (10^3/µl) range 4-13.5	3.40	2.66	0.05	3.40
Neutrophils (10^3/µl) range 1.32 – 7.97	0.90	1.10	-	1.41
Monocytes (10^3/µl) range 0.14-1.48	0	0	-	0.19
Platelets (10^3/µl) range 150-450	191	120	106	139
Hemoglobin (g/dL) range13-16	11.2	9.6	9.1	10.7
C-reactive protein (CRP) (mg/dl)	-	9.5	24.88	-

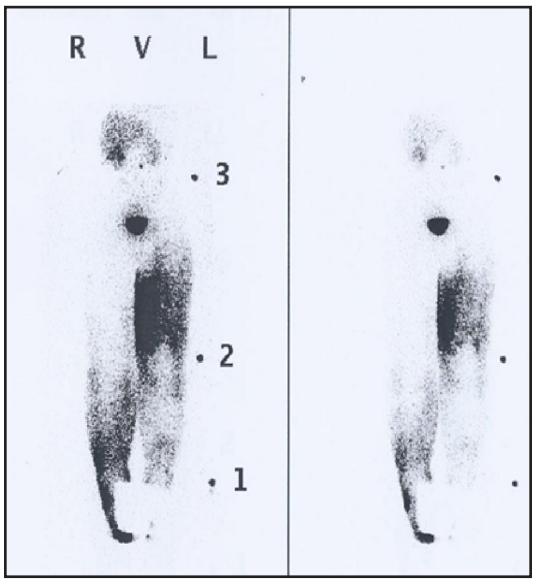

Fig. 2. Lymphoscintigraphy (99mTc-nanocolloid 98 MBq – sc, 3h.30 – 4h) performed in 2009 showed absence of right inguinal uptake and major proximal dermal back-flow in the left lower limb with poor imaging of the left latero-iliac lymph node.

Lymphoscintigraphic examination (*Fig. 2*) provided ambiguous results (absence of right inguinal uptake, major proximal dermal back-flow in the left lower limb with poor imaging of the left latero-iliac lymph node).

Despite repeated physical treatments, from age 5 years the patient began to develop recurrent episodes of lymphangitis, often with erysipelas (high fever, redness of affected limbs, poor general condition, pain) that responded decreasingly to broad spectrum antibiotics. Magnetic resonance lymphangiography of the lower limbs at age 8 years showed diffuse underdevelopment of lymphatic collectors (*Fig. 3*).

Physical treatment was increasingly unable to contain the edema and the patient showed signs of psychological as well as clinical distress. Bone marrow testing at age 9 years showed cell counts and morphology within normal limits. In December 2012, after another episode of erysipelas, bilateral iliac crest biopsy was performed and revealed "low bone marrow cell count with erythroid hyperplasia and megakaryocyte hyperplasia and dysplasia." The histiocyte population was rich and consisted of mature elements and CD68+ cells, sometimes with phagocytic activity.

Lymphangitic episodes became increasingly intense. During one such episode, local acute infection became systemic with septicemic fever that required combined broad spectrum antibiotic therapy in the hospital to save the patient's life. Blood cell counts showed even more severe leukopenia with evidence of major myelodysplasia (WBC 0.05 10^3/µl) (*Table 1*). A mutation in the *GATA2* gene was suspected. In 2014, genetic testing showed a new heterozygous mutation in the *GATA2* gene: c.414_417del, p.(Ser139Cysfs*78) (*Fig. 4*). Subsequent

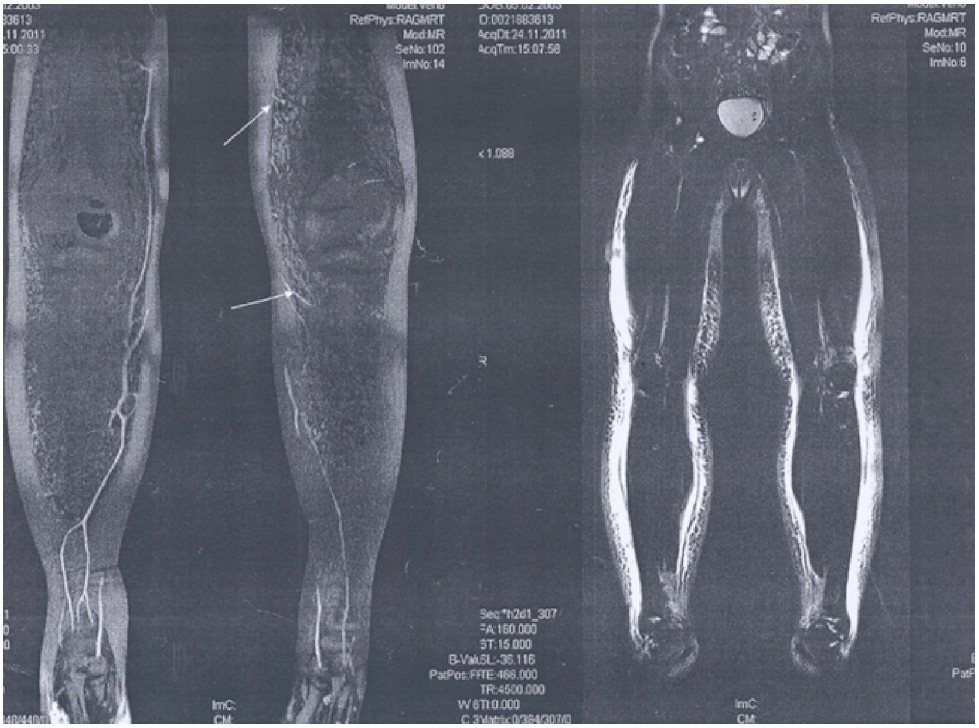

Fig. 3. Magnetic resonance lymphangiography of the lower limbs performed in 2011 showing diffuse underdevelopment of lymphatic collectors.

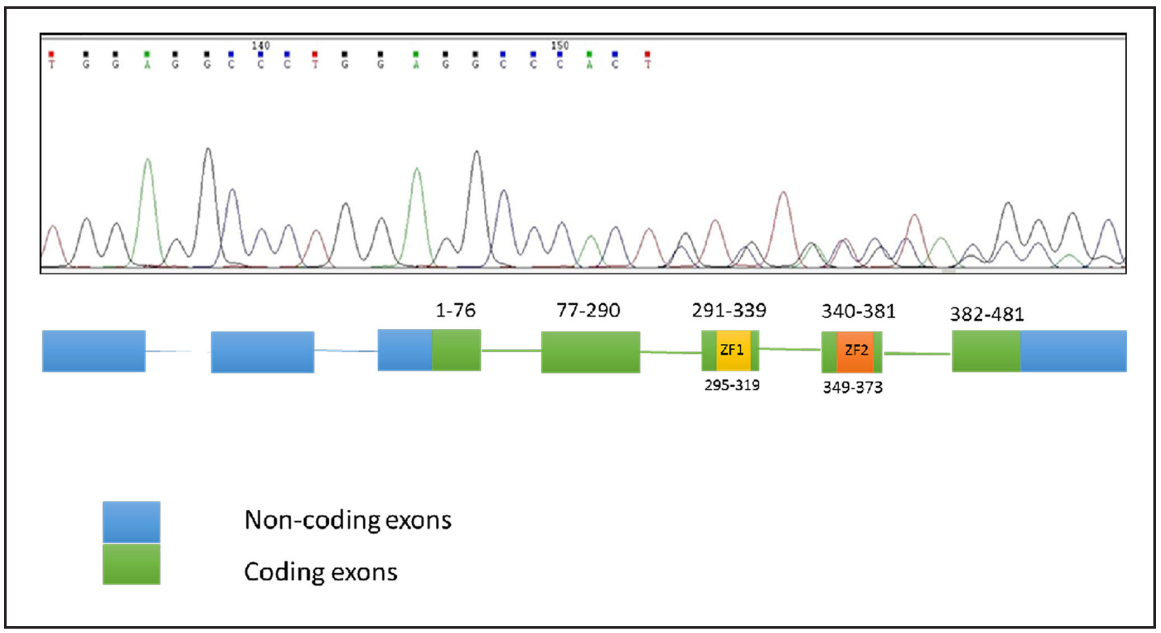

*Fig. 4. Electropherogram showing mutation c.414_417del, p.Ser139Cysfs*78 in the sequence of the GATA2 gene and scheme of amino acid position in the corresponding exon.*

genetic testing of both healthy parents showed that neither carried the son's mutation.

CONCLUSIONS

The positive result of genetic testing confirmed the diagnosis of Emberger syndrome. The patient's pedigree was unremarkable from the viewpoint of Mendelian inheritance with no cases of lymphedema found going back three generations of ancestors and a sister without any suspicious phenotypic characteristics. The *de novo* mutation c.414_417del, p.(Ser139Cysfs*78) in the *GATA2* gene has not been previously described in association with Emberger syndrome or other diseases. Such germinal mutations of *GATA2* appear spontaneously and are then transmitted by autosomal recessive inheritance. On the HGMD website, eight different mutations of *GATA2* associated with Emberger syndrome (or primary lymphedema with myelodysplasia) are reported (3 missense, 2 small deletions and 3 small insertions), and all emerged from the work of Ostergaard et al (4).

It is important to consider that the number of hereditary or sporadic germinal line mutations in the *GATA2* gene reported in the literature is increasing. The mutations have been associated with different phenotypes that overlap in certain characteristics with Emberger syndrome. They include immunodeficiency 21 (OMIM: 614172), acute myeloid leukemia (AML, OMIM: 601626), and myelodysplastic syndrome (MDS, OMIM: 614286) (5). Today we know that all these syndromes are different manifestations of a single genetic disease caused by loss-of-function mutations that give rise to haploinsufficiency of this important hemopoietic transcription factor. A recent study of 57 patients attempted to classify the mutations (missense, null, or regulatory) to identify genotype-phenotype associations. Beyond the vast heterogeneity of clinical manifestations caused by defects in hemopoietic transcription factor *GATA2*, it emerged that lymphedema and viral infections are significantly associated with null mutations like that shown in our report (6). In any case, mutations that induce haploinsufficiency are clearly associated with severe circulating monocytopenia, dendritic cell cytopenia, B and NK lymphocytopenia, and bone marrow failure which can lead to myeloid leukemia (7-9).

Knowing the evolution of the syndrome, the medical staff opted for bone marrow transplant from a parent for treatment. Histocompatibility studies showed good affinity with paternal bone marrow, and the transplant was completed in August 2014 when the patient was 12 years old. In the post-operative period the patient suffered further inflammation and acute congestive heart failure with dyspnea at rest, bilateral basal lung crepitation, anasarca, and high Pro-BNP (> 800 pg/ml), also due to cortisone and immunosuppressive therapy. Diuretic (125 mg furosemide per day) and myocardiokinetic therapy were begun. The patient underwent passive targeted physical therapy and resumed rehabilitation that led to regular use of full-thigh, compression class II elastic stocking under elastic trunks.

In conclusion, this case highlights that in early diagnosis of children with primary lymphedema of the lower limbs and genitalia, it may be advisable to check for both abnormal blood parameters and signs of immune deficiency. The genetic testing for mutations in *GATA2* makes it possible to confirm the clinical diagnosis by determining etiology and provide potential indications for therapy and follow-up in these children.

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Maltese Paolo Enrico, PhD
MAGI Non-Profit Human Medical Genetics Institute
Via Delle Maioliche 57/D
38068 Rovereto (TN), Italy
Fax: 39-0464/425634
Tel.: 39-0464/662771
E-mail: paolo.maltese@assomagi.org