Allogeneic Bone Marrow Transplant: Cure for Familial Mediterranean Fever.

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Section: Regular manuscript

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Abstract:
We describe a seven year-old girl with congenital dyserythropoietic anaemia (CDA), who also had Familial Mediterranean Fever (FMF). Repeated transfusions required since the age of 6 months to treat her CDA led to iron overload and a persistently high ferritin level. Her relapsing FMF made effective iron chelation therapy very difficult. Consequently, she underwent allogeneic, sibling bone marrow transplant (BMT) at the age of 4 years.

During conditioning for her BMT, symptoms of FMF, including splenomegaly, arthritis and recurrent abdominal pain, began to resolve and she was gradually weaned off colchicine. Now, two years post-transplant, she remains free from FMF symptomatology and is off all immunosuppressants.

This case demonstrates that symptoms of FMF can be alleviated by the therapy used during allogeneic BMT. In this patient it is likely that the missing factor in FMF is now being provided by granulocytes derived from the stem cells within transplanted bone marrow.

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INTRODUCTION

Familial Mediterranean Fever (FMF: OMIM 249100) is an autosomal recessive disorder characterised by short, episodic bouts of fever, inflammatory serositis causing arthritis, abdominal pain, pleurisy and pericarditis, and an erysipelas-like erythematous rash. The ESR is classically increased, with the white blood cell count usually being normal. Amyloidosis can be a long-term complication. Normal serosal fluid contains an inhibitor of neutrophil chemotaxis that acts by antagonising the complement-derived chemotactic anaphylatoxin C5a. This inhibitory activity is less than 10% of normal in FMF patients suggesting that C5a-inhibitor deficiency may contribute to the pathogenesis of the inflammatory episodes. Colchicine has proved efficacious in minimising these acute episodes and in preventing the long-term complications of amyloidosis.

The gene responsible for FMF, MEFV, maps to chromosome 16p, and encodes a 791 amino acid protein, variously known as pyrin or marenostrin. The function of this protein, which is predominantly expressed in granulocytes and synovium, remains to be established, but the most recent evidence suggests it may be an interferon- mediated regulator of the inflammatory response. A number of mutations have been described, with certain mutations associated with particular ethnic groups, suggesting a possible heterozygote advantage.

The congenital dyserythropoietic anemias (CDA) are rare inherited disorders affecting the normal differentiation-proliferation pathway of the erythroid lineage. The CDAs comprise a group of heterogenous disorders characterised by ineffective erythropoiesis as the predominant mechanism of anemia and distinct morphological abnormalities of the majority of erythroblasts in the bone marrow. The CDAs have been classified into three types based on morphologic and serologic findings, but there is clearly heterogeneity within the types and many individual cases of CDA have been reported which do not fit into this classification.
We report a patient who had both CDA and FMF, who required allogeneic bone marrow transplantation (BMT) as treatment for the CDA. As a consequence of the BMT she no longer has any symptoms which can be attributed to FMF. We believe this is the first report of treatment of FMF by BMT, which in this patient appears to have been curative.
MATERIALS AND METHODS

Case report

The patient (NK) was the 4th child of consanguineous Coptic Egyptian parents. At the age of 6 weeks she was admitted with a history of irritability and reduced movement of the right arm since soon after birth, with a one-day history of swelling of the right elbow. Her blood count showed a haemoglobin of 57g/l, white cell count of 10 x 10^9/l and platelets 795x 10^9/l. Her blood film showed a leucoerythroblastic picture and a subsequent bone marrow examination showed features typical of congenital dyserythropoietic anaemia type II. There were no sideroblasts.

NK required further hospital admissions aged 7 weeks, 10 weeks, 6 months and 11 months with recurrent episodes of joint swellings associated with fever and marked elevation of C-reactive protein and ESR. At 6 months she was admitted with pericarditis, followed by further admissions with episodic vomiting, lethargy, poor feeding, diarrhoea and hepatomegaly. A presumptive diagnosis of FMF was made aged 14 months by one of us (A.M.), and she was commenced on colchicine with an improvement in her symptoms.

Despite the regular colchicine therapy she continued to suffer frequent relapses, with abdominal pain, arthritis, fever and splenomegaly. Her mother would increase her dose of colchicine during these attacks. On four occasions these relapses were sufficiently severe to warrant admission, once leading to a laparotomy for suspected small bowel obstruction.

Her chronic ill health was associated with moderate global developmental delay and failure to thrive. She also had complex feeding difficulties necessitating nasogastric tube feeding from the age of 10 months.

To treat her anaemia NK required several blood transfusions from 6 months, progressing to regular transfusions after 12 months. She developed a rising ferritin level and was commenced on iron chelation therapy from 3 years 7 months, with a desferroxamine pump six days out of seven. Compliance with this
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regimen became increasingly difficult due to her recurrent episodes of vomiting, diarrhoea and arthritis. Ten months after chelation therapy started her ferritin level was 10 673 microgram/l (normal range; 10 – 150). Her mother felt that her symptoms of FMF were frequently exacerbated by the desferroxamine, and so compliance with this remained poor. Her general health was not improved by transfusion. A liver biopsy performed six months prior to BMT showed no cholestasis, steatosis or significant fibrosis, but with a liver iron level of 180.5 micromoles/g (normal range < 30 micromole/g).

Tissue typing showed that she had a matched sibling donor. Molecular typing showed identical A, B and DRB1 markers. The Helper T-lymphocyte precursor frequency was 1: 1,081,147, suggesting a very low risk of graft versus host disease (GvHD)13.

Mutation analysis

Genomic DNA was extracted from peripheral blood samples taken from patients and controls using standard techniques14.

Screening for the “Common” Mutations

The fragment encompassing the common mutations in exon 10b (M680I, Δ692I, M694V, M694I, V726A, and R761H) was amplified using “hot start” PCR15 and the oligonucleotide primers described by the International FMF Consortium5. The cycling conditions used were: initial denaturation step- 95°C for 10 minutes, followed by 40 cycles of 95°C for 30 seconds, 63°C for 30 seconds, 72°C for 30 seconds, and a terminal elongation step at 72°C for 4 minutes. The amplification was performed in a total of 25µL composed of 2.5µL 10X PCR buffer II (Perkin Elmer), 15pmol of each primer, 0.16mM dNTP solution, 1U ampliTaq Gold (Perkin Elmer), 2.0mM MgSO₄ solution and DNA 100ng.

The presence of the V726A mutation (Alu I site created), the M694V mutation (Hph I site created) and the M680I mutation (Nla III site destroyed) were analysed by digesting 5µL of the PCR product at 37°C overnight with 2-2.5U of appropriate restriction enzyme (New England BioLabs) and digestion buffer as
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per the supplier's specifications to a final volume of 20µL. The other mutations were screened by automated sequencing of the exon 10b PCR product (SUPAMAC, University of Sydney).

**DHPLC Screening**

The rest of the coding sequence was screened by denaturing high performance liquid chromatography (DHPLC), using the Prostar Helix System (Varian Inc). Exons 1, 3, 4, 5, 6, 7, and 10a were amplified using the oligonucleotide sets from the International FMF Consortium. Exon 2 was amplified using the oligonucleotide primers described by Aksentijevich. Exons 8 and 9 were amplified with the following oligonucleotides: exon 8; 8 forward CTCAGGATAGATGGGCTTGG, 8 reverse CAGCACAAGGGAACACTGC, exon 9; 9 forward GACTCATGAGACACAGTG, reverse primer for exon 8/9 from the International FMF Consortium. Heteroduplexes were formed by denaturing the PCR products at 95°C for 10 min then slowly cooling to 55°C over 30 min.

The theoretical temperature for each PCR product was determined from [http://insertion.stanford.edu/melt.html](http://insertion.stanford.edu/melt.html) and then optimised for temperatures +/- 2°C the predicted temperature. The optimal temperature was determined by comparing the retention time of the same sample over the five different temperatures and on peak morphology. The temperature chosen was either one where a heteroduplex was seen or the temperature at or before the peak started to broaden, in cases where this was difficult to determine two temperatures were used. The temperatures used were as follows: Exon 1 (63°C), 2a (69°C), 2b (69°C), 3 (63°C), 4 (61°C), 5 (61°C and 62°C), 6 (59°C and 60°C), 7 (61°C), 8 (60°C), 9 (60°C and 61°C) and 10a (59°C and 60°C). Where a heteroduplex was identified, the presence of a sequence variation was confirmed by automated sequencing (AGRF, University of Queensland).

Where no heteroduplex was identified in an amplicon, that amplicon was subjected to automated sequencing. Thus, the whole of the coding region was screened by DHPLC and automated sequencing.
RESULTS

Bone Marrow Transplantation

NK was admitted one month prior to the planned BMT for two weeks intravenous chelation therapy, bringing her ferritin level down from 5700µg/l to 3425µg/l. Her main symptoms of FMF at this stage were swelling of her left elbow, splenomegaly and diarrhoea.

Conditioning therapy, which was commenced at four years of age, consisted of 4 doses of busulphan, 150mg/m²/day (6.3mg/kg/day) as a single daily dose on days –9 to –6; 4 doses of cyclophosphamide, 50mg/kg/day on days –5 to –2, and 3 doses of anti-thymocyte globulin, 15mg/kg, on days –8, -6 and –4. GvHD prophylaxis was with cyclosporin, initially at 5mg/kg/day from day –1. Unmanipulated bone marrow was reinfused on day 0, with a nucleated cell count of 6 x 10⁸/kg recipient body weight. The donor was her well brother, who was clinically unaffected by either condition.

The post-transplant course was uncomplicated. NK tolerated nasogastric feeds well throughout the BMT. She received one course of antibiotics for a *Klebsiella pneumoniae* septicemia. She developed clinical skin GvHD on day +38, which was treated with oral prednisolone from days +38 to +93. She showed full haematological recovery with neutrophil count of 0.5 and 1.0 x 10⁹/l on day +21 and platelets of 25, 50 and 100 x 10⁹/l on days + 45, 50 and 87. Her last transfusion of packed cells was on day + 9, and she has been undergoing regular venesection since day + 66. Her last serum ferritin was 207 µg/L at 28 months post-BMT and no further venesections are planned.

Donor chimerism was confirmed by analysis of PHA-stimulated lymphocytes from peripheral blood. The first successful analysis was on day + 73, when 2/30 cells were female. All subsequent analyses, from day +108 to two years post-BMT revealed male cells only. Cyclosporin was ceased at 8 months post-BMT.

Course of symptoms of FMF post-BMT
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The patient’s symptoms of FMF rapidly abated during the conditioning therapy and colchicine was ceased the day after BMT. Her persistent left elbow effusion resolved and her splenomegaly rapidly disappeared. She remains free of any symptoms of FMF at 28 months post-BMT, twenty months after cyclosporin therapy was ceased. At fourteen months post-BMT, she had an episode of Enterobacter septicaemia. This was associated with fever and transient splenomegaly, but no other FMF symptoms. NK is feeding well orally and has started kindergarten. She has shown significant catch-up growth, with an increase in height from a SDS score of –3.55 at BMT to –1.83 at 26 months post-BMT. Her weight has increased from 19th to 50th percentile (SDS of –0.89 to –0.0).

Mutation analysis

DNA extracted from peripheral blood samples was analysed pre-BMT for the patient and at 10 months post-BMT for the patient and donor. In the patient’s DNA collected pre-BMT, one allele was found to have the M680I mutation by Nla III restriction digest and direct sequencing (Figure 1). However, despite DHPLC screening and sequencing of the whole coding region, no other disease-causing mutation was identified.

In addition, the patient was found to be heterozygous for the following silent polymorphisms: A414G (G138; exon 2), C495A (A165; exon 2), C942T (R314; exon 3), A1422G (E474; exon 5), G1428A (Q476; exon 5) and T1530C (D510; exon 5) [results not shown].

Her clinically normal donor brother did not have the M680I mutation. He was also found to have the six silent polymorphisms, and parental studies revealed that the 6 polymorphisms were inherited from their mother, whilst the M680I mutation was inherited from their father [results not shown].
DISCUSSION

Whilst the precise function of pyrin/marenostrin remains to be established, available evidence suggests that it plays a role in the regulation of inflammatory processes\(^\text{16}\). Recently, it was shown that a pyrin isoform, which has an in-frame deletion of exon 2, shows a markedly different subcellular localisation pattern to the full length protein in transient expression studies, with spliced variant targeted mainly to the nucleus whilst the full-length form localised to the cytoplasm\(^\text{7}\). Based on these results, it has been speculated that the different mutations in the \textit{MEFV} gene might have different biological consequences\(^\text{7}\). It would be premature at this stage, however, to speculate whether the combination of \textit{MEFV} mutations in NK had a pathogenetic role her development of CDA.

Congenital dyserythropoietic anemia type II (as in this case) is the most frequent form of congenital dyserythropoiesis. It is characterised clinically by an anemia of variable severity, jaundice and hepatosplenomegaly. Gall bladder disease and secondary haemochromatosis are frequent complications\(^\text{17}\). Erythroid hyperplasia with binuclearity or multinuclearity involving late erythroblasts in the bone marrow is a key feature of the diagnosis. In addition, the CDA type II erythrocytes display antibody-mediated sensitivity to lysis in acidified sera of many ABO compatible donors, which has lead to the alternative name of HEMPAS (\textit{Hereditary Erythroblastic Multinuclearity with Positive Acidified Serum})\(^\text{18}\). The erythrocyte membrane proteins show abnormalities of glycosylation, in particular band 3, the anion exchanger\(^\text{19}\). Underglycosylated band 3 aggregates in solution and clusters in membranes, resulting in membrane disorganization\(^\text{20}\). Evidence has been provided for the localisation of the CDA II locus to chromosome 20q11.2 by linkage analysis, although at least 5 –10% of cases failed to map to 20q stressing genetic heterogeneity\(^\text{21}\). In a few patients the underglycosylation of band 3 has been identified as resulting from a deficiency in the activity of, either \(\beta\)-4-galactosyltransferase (GalT), N-acetylgalcosaminyltransferase (GnT II) or \(\alpha\)-mannosidase II (Man II). However none of the genes for these enzymes localise to chromosome 20\(^\text{22}\), suggesting that these deficiencies may be secondary changes, or alternatively result from a deficiency of a tissue-specific transcription factor. Thus, despite progress in the field the primary molecular defect responsible for CDA type II remains unknown.
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There has previously been one report of a family with three affected members (two brothers and a cousin) who experienced CDA in association with chronic recurrent multifocal osteomyelitis (CRMO) and Sweet syndrome\(^{23}\). Interestingly this family was of a consanguineous Palestinian Arab background. The authors proposed that the inherited tendency to the two disorders segregated together. Because of this report a diagnosis of CRMO was entertained in our patient early in the course of her disease when she had predominantly bone and joint inflammation. It is interesting to speculate that perhaps the patients in this report in fact had FMF and not CRMO, and perhaps the FMF and CDA segregate together. Alternatively the CDA or the defects of glycosylation may have an impact on the FMF phenotype.

A disease-causing mutation was identified in only one allele of the proband, whilst the other allele harboured 6 apparently silent polymorphisms, which do not coincide with any of the previously reported founder haplotypes\(^{24}\). It is possible that the pathogenic mutation in the other allele is located in the promoter region of the gene, or in an intronic region not sequenced which might affect splicing of the mRNA. Another possibility is that one of the apparent silent exonic polymorphisms affects an exonic splicing enhancer, resulting in perturbations of the normal splicing processes\(^{25,26}\). It is also possible that in this family the FMF trait has been transmitted with true autosomal dominant inheritance, as has been recently reported\(^{27,28}\), although this has not been observed to date for the M680I mutation. Finally, it may be that together the combination of silent polymorphisms in \textit{cis} alters the structure or function of the pyrin polypeptide in a deleterious manner. Confirmation or exclusion of these possibilities may be possible after the development of a functional assay for the pyrin gene product and mRNA studies.

Bone marrow transplantation, once confined to the treatment of haematological malignancies abnormalities\(^{29,30}\), has now expanded to treat other stem cell and other monogenic disorders\(^{31-33}\). Clearly the potential risks of BMT would not routinely be justified in the treatment of FMF, particularly...
when there is an effective, simple and relatively safe treatment in the form of colchicine for both acute attacks and long-term complications.

It is unclear whether the patient’s haematological condition exacerbated her FMF, but her parents had the distinct impression that desferroxamine therapy worsened her symptoms, making compliance difficult. Because of severe iron overload, BMT became mandatory to treat the CDA, with the fortuitous result that the BMT appears to have also “cured” her FMF, at least after 28 months of observation off colchicine post-BMT, and 20 months off all immunosuppressants.

We believe that this is the first reported case of a patient with FMF receiving a BMT. We suggest that BMT should be considered, albeit as a last resort, in patients who are extremely unresponsive to all therapies including colchicine and interferon-\(\alpha\)\textsuperscript{34}. 
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References:
5. The International FMF Consortium: Ancient missense mutations in a new member of the RoRet gene family are likely to cause Familial Mediterranean Fever. Cell 90:797 - 807, 1997


Figure Legends:

**Figure 1:**

**Identification of M680I (G2040C) mutation.** a) A 391 bp PCR fragment was digested with *Nla III*. The normal allele is cut to 320, 46 and 25 bp fragments, whereas the mutation (which leads to a loss of a *Nla III* site) results in a 366 and 25 bp fragments. b) This was confirmed by automated sequencing.
a) NK normal

![Genetic sequence image with bands at 404bp, 331bp, 366bp, and 320bp]

b) CATG to CATC

![Genetic sequence image with comparison to normal sequence]
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