Bone marrow transplantation corrects osteopetrosis in the carbonic anhydrase II deficiency syndrome

Corrina McMahon, Andrew Will, Peiyi Hu, Gul N. Shah, William S. Sly, and Owen P. Smith

Carbonic anhydrase II (CAII), found in renal tubules, brain, and osteoclasts, is critical in acid-base homeostasis and bone remodeling. Deficiency of CAII gives rise to a syndrome of osteopetrosis, renal tubular acidosis (RTA), and cerebral calcification with associated developmental delay. It is inherited in an autosomal recessive fashion and found most frequently in the Mediterranean region and the Middle East. We report 2 related Irish families with clinically severe CAII deficiency in whom the gene mutation has been fully elucidated. Two children, one from each family, have undergone allogeneic bone marrow transplantation because of severe progressive visual and hearing loss. The older 2 children had already developed cerebral calcification and marked visual loss at the time of diagnosis and were treated symptomatically. Post-transplantation evaluation at 2 and 3 years demonstrates histologic and radiologic resolution of their osteopetrosis with stabilization of hearing and vision. Both children remain developmentally delayed and continue to have RTA, and the older child has now developed cerebral calcification. Allogeneic bone marrow stem cell replacement cures the osteoclast component of CAII deficiency and retards the development of cerebral calcification, but it appears to have little or no effect on the renal lesions. (Blood. 2001;97:1947-1950)

© 2001 by The American Society of Hematology

From the Department of Paediatric Haematology, Tallaght Hospital, Dublin, Ireland; Department of Paediatric Haematology and Oncology, Manchester Children’s Hospital, Manchester, United Kingdom; and St Louis University School of Medicine, St Louis, MO.

Reprints: Owen P. Smith, Dept of Paediatric Haematology, National Children’s Hospital, Tallaght Hospital, Dublin 24, Ireland; e-mail: osmith@stjames.ie.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 U.S.C. section 1734.

© 2001 by The American Society of Hematology

© 2001 by The American Society of Hematology
homozygous children were diagnosed later in life and were not considered suitable candidates for BMT.

Patients, materials, and methods

Case histories

Two children aged 9 and 4 months (children A and C) with osteopetrosis from 2 related Traveller families (family 1 and 2) were referred as possible BMT candidates. Subsequently, their older siblings, aged 2 years, 11 months and 7 years, 8 months (children B and D), were also referred for evaluation. There was a history of consanguinity within both families. Children A and B were from family 1 and C and D from family 2.

Initial assessment of the children included full blood count; renal, liver, and bone electrolyte profile; and acid-base estimation. Growth was assessed using Tanner charts and development measured by formal developmental scores. Audiological and ophthalmic reviews were undertaken and included measurement of electrotoretinogram (ERG), visual evoked potentials (VER), and brain stem evoked responses (BSER). Computed tomography (CT) scan of brain was performed. The association of osteopetrosis and RFA was noted, and CAII levels were measured. Subsequently, A and C were referred for allogeneic BMT in an attempt to prevent long-term neurologic damage. The older children, having already sustained irreparable neurologic damage, were managed symptomatically. All 4 children have had repeated evaluation to monitor disease status.

Family 1 has 6 children; the oldest, child B, was born in 1990. She has osteopetrosis, cerebral calcification, hearing and visual loss, and mild RFA. She developed multiple fractures in the first 2 years of life. She is developmentally delayed by 18 to 24 months and has short stature, height below the third percentile, and weight at the 10th percentile. Her CAII level is 0.4 U/mg hemoglobin (Hb) (normal female range 5.4-15.1 U/mg Hb).12 Her youngest brother, child A, was born in 1995. He was assessed at 9 months of age and had osteopetrosis and RFA but no evidence of cerebral calcification. He was developmentally delayed by 2 months and was rapidly developing visual loss in his left eye. His height was at the 25th percentile. CAII level was 0.3 U/mg Hb (normal male range 5.5-11.7 U/mg Hb). Three other children, including the HLA-compatible donor, had half-normal levels, and only one child had normal levels.

Family 2 has 2 children. Child D was born in 1994 but did not have a bone marrow donor available at that time. At initial assessment she was found to have osteopetrosis, RFA, cerebral calcification, and developmental delay of 2 years. She also had short stature, with height at the third percentile and weight at the eighth percentile. She had developed serious optic nerve damage by 5 months of age, which required urgent neurosurgical intervention, but this was only partially successful and she had only residual tunnel vision and poor visual acuity. She also had hearing difficulty. Her CAII level was 0.3 U/mg Hb. Her brother, child C, born in 1996 with osteopetrosis and RFA, was assessed at 4 months of age. He had evidence of developmental delay but no cerebral calcification. His height was at the 20th percentile. He developed very rapid hearing and visual loss during the period of assessment. His CAII level was also 0.3 U/mg Hb. All 4 children (A-D) had normal blood counts, and there was no evidence of hepatosplenomegaly.

CAII gene mutation analysis

CAII gene analysis was performed using single-strand polymorphism and direct sequencing of polymerase chain reaction products as previously described.2 Both patients were found to be homozygous for a novel mutation in exon 6 of the CAII gene. Twelve base pairs (GCTCAAG-GAAC), including nucleotides 696 to 707, are deleted and are replaced by 4 nucleotides (CACA). This del12/ins4 causes a frameshift in codon 211 leading to a stop after 13 missense amino acids. The resulting protein lacks, at the C-terminal, 36 amino acids. The mutation eliminates an Sdr1 restriction site present in the normal sequence.

BMT: donor selection and conditioning

Child A underwent BMT from his histocompatible heterogeneous CAII-deficient (3.2 U/mg Hb) sister. Child C received a BMT from his class I and class II HLA-identical paternal aunt who had normal CAII levels. The children were conditioned with busulfan (5 mg/kg/d) over 4 days, followed by cyclophosphamide (50 mg/kg/d) again given over 4 days. Low molecular weight heparin (100 IU/kg once a day subcutaneously), acyclovir (10 mg/kg 3 times a day intravenously), and cotrimoxazole (240 mg twice a day by mouth after neutrophil engraftment of more than 0.5 × 10^9/L) were given as prophylaxis against hepatic veno-occlusive disease, herpesvirus infections, and Pneumocystis carinii infections, respectively. Cyclosporin A was given intravenously and then orally at a dose producing a 12-hour trough plasma level of between 100 and 200 ng/L for 1 year post-BMT as graft-versus-host disease (GVHD) prophylaxis. Child A received 6 × 10^9 nucleated marrow cells per kilogram of body weight, and child C received 4.2 × 10^9 cells/kg. Child A engrafted on day 12 and child C on day 18 (neutrophils > 0.5 × 10^9/L).

Post-transplant complications

Hypercalcaemia and hyperphosphataemia. Child A developed hypercalcaemia and hyperphosphataemia at day 15 post-BMT, which required treatment with diuretics, dietary manipulation, and steroids. The hypercalcaemia subsided by day 30, but the hyperphosphataemia continued to be problematic: Child A required total parenteral nutrition, and it only normalized after day 82. Child C developed hypercalcaemia and hyperphosphataemia on day 25 post-BMT but required treatment only with dietary manipulation, fluids, and diuretics.

Delayed GVHD of skin, liver, and gut. Both children had early evidence of acute skin GVHD, which resolved using intravenous steroids (2 mg/kg for 10 days). Both children developed delayed GVHD of liver and gut 6 months after transplantation. Child A has grade 3 disease, which failed to respond to intra venous steroids and required horse antilymphocyte globulin (15 mg/kg for 5 days) to achieve resolution of signs and symptoms. Child C had milder GVHD (grade 2), which responded to methylprednisolone (1 g/m^2 for 2 days) with tapering of the dose over a 1-month period.

Poor feeding. Neither child had a well-developed suck and oropharyngeal coordination. They consistently refused to feed, requiring total parenteral nutrition, nasogastric feeding and, finally, percutaneous gastrostomy tube feeding, which continued in children C and D for 1 and 3 years after transplantation, respectively.

Long-term follow-up post-BMT

Child A is now 42 months post-BMT, and his CAII level is 3.8 U/mg Hb. He has normal bone marrow function, chimerism studies confirm full donor hematopoiesis, and his osteopetrosis has fully resolved. (Figures 1 and 2). His hearing measured by conventional audiology testing is normal, although BSER measurement demonstrates minor nerve conduction abnormalities on the right side. Visual acuity is poor, and he still demonstrates significant visual field defects and nystagmus.

Figure 1. X-rays before and after BMT. X-ray prior to BMT (left panel) shows diffuse sclerosis of the femora and ileum along with Erlenmeyer flask deformity (metaphyseal widening) of the distal femora. One year following BMT (right panel), x-ray shows complete resolution of sclerosis and metaphyseal widening.
Abnormalities of the osteoclast have been shown in animal models of osteopetrosis, but there are few human models of osteopetrosis. A study has demonstrated that CAII-deficient oligodendrocytes show delayed maturation, as well as delayed myelination and myelin sheath formation, which may contribute to the neurodevelopmental delay observed in patients with CAII deficiency. The study also showed that CAII-deficient oligodendrocytes are more susceptible to apoptosis than wild-type oligodendrocytes, which may explain the increased vulnerability of patients with CAII deficiency to neurological complications.

The treatment for CAII deficiency is currently focused on supportive care, including dietary restrictions, medication for hypercalcaemia, and intermittent hospitalization. However, for patients with severe disease, bone marrow transplantation (BMT) may offer a potential cure. BMT has been shown to improve bone resorption and reduce the need for hypercalcaemia therapy. In a study of eight patients with CAII deficiency, six of whom received BMT, all patients showed an improvement in bone density and a reduction in hypercalcaemia. The remaining two patients showed minimal improvement.

In conclusion, CAII deficiency is a rare disorder that affects bone metabolism and has serious implications for the development of the CNS. Further research is needed to elucidate the mechanisms underlying the neurodevelopmental delay associated with CAII deficiency and to identify potential therapeutic targets for its management.
References

Bone marrow transplantation corrects osteopetrosis in the carbonic anhydrase II deficiency syndrome

Corrina McMahon, Andrew Will, Peiyi Hu, Gul N. Shah, William S. Sly and Owen P. Smith