Homozygous AMN mutation in hereditary selective intestinal malabsorption of vitamin B_{12} in Jordan

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ABSTRACT

Objectives: Juvenile megaloblastic anemia is a rare and often hereditary disorder of cobalamin absorption, transport or intracellular metabolism. Several syndromes present with megaloblastic anemia such as congenital megaloblastic anemia due to intrinsic factor defect and juvenile megaloblastic anemia with proteinuria due to defects in the cubilin or the amnionless protein.

Methods: We identified a large kindred with juvenile megaloblastic anemia. Four genes, GIF, CUBN, TCN1, and TCN2, was previously excluded from being responsible for the syndrome of this family who was discovered in Irbid, Jordan, during the year 1999. At that time, the amnionless (AMN) gene was not yet known to implicate in megaloblastic anemia. In this study, we screened the AMN for mutations in the Ohio State University, Iowa, United States of America. In addition, follow-up testing was carried out in the University of Iowa in 2004.

Results: We identified a homozygous splice site mutation in the patients. This mutation was previously detected in families from Turkey and Tunisia. It is suspected to be a founder mutation of Middle Eastern origin.

Conclusion: Molecular testing for this specific mutation in cases of Middle Eastern origin is a valuable tool for presymptomatic diagnosis, carrier identification and perhaps prenatal diagnosis.

Saudi Med J 2005; Vol. 26 (7): 1061-1064

Megaloblastic anemia during infancy and early childhood is quite rare and often reflects a hereditary disorder of cobalamin metabolism. All known inborn errors of cobalamin metabolism are autosomal recessive disorders with megaloblastic anemia being a constant feature, while each disorder displays further characteristics specific for the underlying defect.[1] Inborn cobalamin errors usually result from defects in its absorption, transport, or intracellular metabolism.[1] The metabolic pathway of dietary cobalamin starts by its binding to a salivary R-binder protein which is cleaved off in the proximal small intestine by pancreatic enzymes.[1] Cobalamin is then bound to the gastric intrinsic factor (IF), encoded by GIF, forming a complex that attaches to a specific surface receptor, cubilin which is encoded by CUBN, at the mucosa of the distal ileum.[2] The amnionless protein, encoded by AMN, binds to cubilin and aids in its function.[3,4] The cubilin and amnionless protein complex transports cobalamin across the membrane by endocytosis.[3] While the receptor complex is recycled, the IF is

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Received 26th December 2004. Accepted for publication in final form 30th April 2005.

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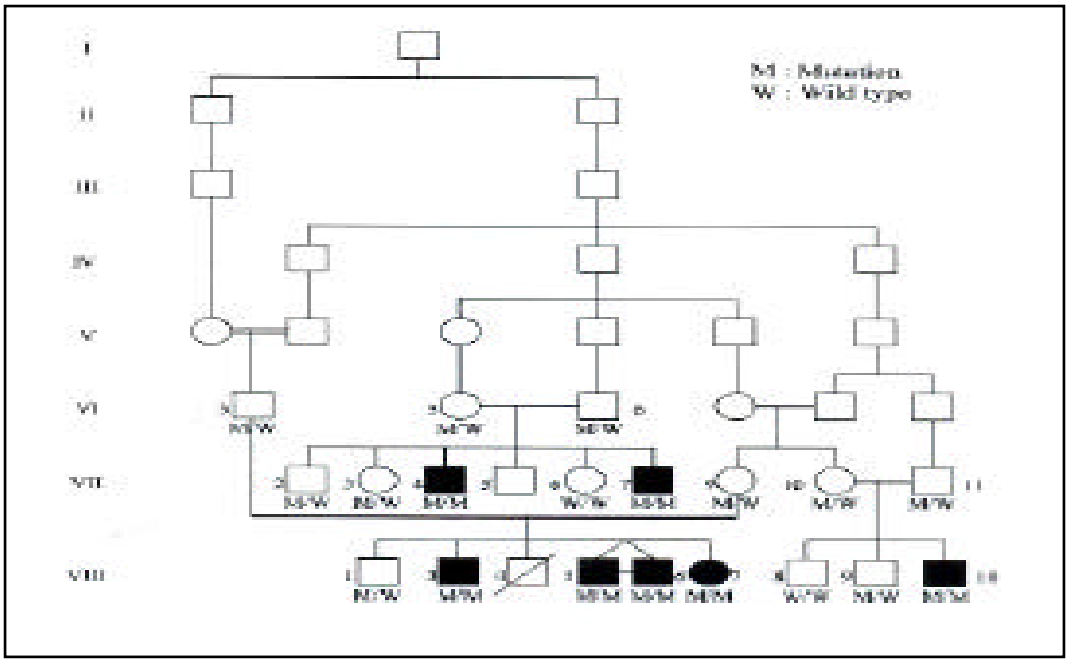

Figure 1 - The pedigree of the family showing the genotype of each tested individual regarding the mutation c.208-2A→G. M stands for mutation; W stands for wild type.

degraded in lysosomes and the cobalamin is released into the bloodstream. Once in the portal compartment, cobalamin is delivered to cells by transcobalamin I encoded by TCN1 and transcobalamin II, encoded by TCN2.[5] The transcobalamin-cobalamin complex attaches to a receptor that facilitates the entry of cobalamin into the cell by absorptive endocytosis.[6] Inside the cell, cobalamin is prepared for the execution of its co-enzymatic role either by methylation or adenosylation.[1]

There are several well-described autosomal recessive syndromes with megaloblastic anemia that are due to defects in cobalamin absorption or transport. These include congenital pernicious anemia (MIM # 261000),[7] congenital megaloblastic anemia with proteinuria (MIM # 261100) also known as Imerslund Gräsbeck syndrome[8,9] and transcobalamin II deficiency (MIM # 275350).[10]

We recently reported a highly inbred Jordanian family with autosomal recessive megaloblastic anemia, presenting during infancy, that was completely reversed by parenteral vitamin B_{12} therapy, and in whom four genes, GIF, CUBN, TCN1 and TCN2, were excluded by linkage analysis.[11] Herein, we report that a splice site mutation in AMN is responsible for the syndrome in the family when homozygous.

Methods. We identified a large inbred pedigree in which multiple sibs in several sibships had megaloblastic anemia from Irbid, Jordan in 1999 (**Figure 1)**. They represent a subset of a larger extended family (clan) that inhabits 3 villages in Northern Jordan and in whom childhood-onset megaloblastic anemia is diagnosed with considerable frequency. The clinical features of this family was previously describe Al-Alami et al.[11] Involvement of GIF, CUBN, TCN1 and TCN2 in the etiology of this disorder in the family was excluded previously.[11]

Mutation analysis and screening. Amplification of the AMN exons and their splice sites was performed from genomic DNA, isolated from peripheral blood in the Ohio State University as described.[4] The PCR amplification products were run on an agarose gel, and the bands containing the amplified products were recovered and extracted from the agarose by column purification. The amplified products were sequenced using an automated ABI sequencer with dye-terminator chemistry. Sequencing was performed in an obligate carrier, an affected individual, and an unrelated control. We screened 200 ethnically matched Jordanian controls for the c.208-2A→G mutation by single-strand conformation polymorphism (SSCP) analysis in the University of Iowa. The amplified fragment was run on a fan-cooled MDE gel for SSCP analysis at 6 W for 14 hours and then silver-stained. The alleles were scored by comparison to a positive control (homozygous affected), an obligate carrier (heterozygote) and a

negative control (homozygous wild type) run with every PCR reaction and on every gel. Any suspicious shift, whether similar or not to the controls, was verified by sequencing. The mutation analysis and screening was carried out in 2004.

Results. We directly targeted the AMN since the AMN mutation c.208-2A→G is a Middle Eastern founder mutation.[12] Indeed, the c.208-2A→G change that causes skipping of exon 4 and a subsequent reading frame shift,[4] was homozygous in all patients and heterozygous in all obligate carriers of the family. The mutation segregated with the phenotype in an autosomal recessive pattern and was not identified in any of the 200 controls. We obtained DNA from an affected individual from the same extended family, but originates from a different village (not shown in the pedigree), and identified the mutation in a homozygous state.

Discussion. The assumption that the megaloblastic anemia occurring in affected individuals in this family is related to a metabolic cobalamin defect is based on several facts; the most important being the curative effect of parenteral vitamin B_{12} therapy. The inconsistent presence of proteinuria in some patients, even on treatment, is suggestive of Imerslund-Gräsbeck syndrome, but is not conclusive for the final diagnosis. It was difficult to exclude congenital pernicious anemia based on clinical grounds only. The Schilling test could not be performed in Jordan due to hazards of radioactivity. The relatively mild clinical picture, the absence of other clinical findings, the low vitamin B_{12} serum levels, and the age at presentation together suggested the exclusion of transcobalamin II deficiency. Later, AMN was identified as a gene that plays a role in the etiology of Imerslund-Gräsbeck syndrome, as homozygous mutations were found in patients coming from several families .[4,12]

Genetic testing in the family revealed the presence of the AMN mutation c.208-2A→G that leads to aberrant skipping of exon 4,[4] proving that this is a case of Imerslund-Gräsbeck syndrome. The Middle Eastern founder mutation was previously documented in Turkish and Tunisian Jewish families.[12] It has been hypothesized that during the long history of the Ottoman Empire, this mutation may have drifted from Tunisia to Turkey or the reverse. We can now add the inhabitants of Northern Jordan to the populations affected by this founder mutation. Although this addition does not resolve the issue of the geographic origin of this mutation, it demonstrates its widespread distribution in the Middle East, specifically in the Mediterranean populations. These conditions are reminiscent of the MEFV mutations, responsible for the familial Mediterranean fever phenotype, which is common in Sephardic Jews, Arabs, Turks, and Armenians.[13] It is noted that the Jordanian family described here has related branches in Southern Jordan and Egypt, dating back to 200 or more years, during the Ottoman Empire rule of the region. This might indicate a more extended distribution of this mutation in certain Arabic populations.

The identification of this mutation and its genetic testing provides a tool that can be used diagnostically for putative patients with juvenile megaloblastic anemia from Jordan and other Middle Eastern countries. Testing should be utilized for screening high-risk infants before the development of symptoms avoiding some of the medical complications that might occur due to delay in the institution of the appropriate therapy. In addition, carrier identification and subsequent calculation of risks of having children with this disorder can be accomplished. Although the test could be employed for prenatal testing, the mild and treatable nature of the disorder probably does not justify such testing.

Acknowledgment. The authors wish to thank the family members for their participation in this study. This study was supported in part by funds from the higher counsel of Science and Technology, Amman and Jordan University of Science and Technology, Irbid, Jordan. It was also supported by start-up funds from University of Iowa and Carver College of Medicine, Iowa City, Iowa, USA. Dr. H. El-Shanti was supported by the "Chaire Internationale de Recherche, Blaise Pascal, de l'etat et de la Règion d'Ile-de-France" which is managed with further support by the "Fondation de l'Ecole Normale Supèrieure" during the initial linkage studies. The study was supported by grant CA16058 from the National Cancer Institute, USA.

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