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ORIGINAL ARTICLE

Genetic heterogeneity of megaloblastic anaemia type 1 in Tunisian patients

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Abstract Megaloblastic anaemia 1 (MGA1) is a rare autosomal recessive condition characterized by selective intestinal vitamin B12 malabsorption and proteinuria. More than 200 MGA1 patients have been identified worldwide, but the disease is relatively prevalent in Finland, Norway and several Eastern Mediterranean regions. MGA1 is genetically heterogeneous and can be caused by mutations in either the cubilin (*CUBN*) or the amnionless (*AMN*) gene. In the present study we investigated the molecular defect underlying MGA1 in nine Tunisian patients belonging to six unrelated consanguineous families. Haplotype and linkage analyses, using microsatellite markers surrounding both *CUBN* and *AMN* genes, indicated that four out of the six families were likely to be linked to the *CUBN* gene. Patients from these families were screened for the Finnish, Mediterranean and Arabian mutations already published. None of the screened mutations could be detected in our population. One family showed a linkage to *AMN* gene. Direct screening of the *AMN* gene allowed the identification of the c.208-2A>G mutation, previously described in a Jewish Israeli patient of Tunisian origin and in Turkish patients. This suggests that the c.208-2A>G mutation may derive from a single Mediterranean founder ancestor. For the last family, haplotype analysis excluded both *CUBN* and *AMN* genes, suggesting the existence of a third locus that may cause MGA1.

Keywords Megaloblastic anaemia · Imerslund-Gräsbeck syndrome · Haplotype analysis · Consanguinity · Genetic heterogeneity · Mutation screening

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Introduction

Megaloblastic anaemia in children is mainly due to folate or vitamin B12 (Vit B12) deficiency. Inherited causes of Vit B12 malabsorption can be related to (1) a lack or low secretion of gastric intrinsic factor (IF)

(known as pernicious anaemia, MIM 261000), or (2) a deficiency of transcobalamin II (known as TCII deficiency, MIM 275350), or (3) a defect in selective uptake of the intrinsic factor–vitamin B12 (IF-Vit B12) complex in the terminal ileum (known as megaloblastic anaemia 1, MGA1, MIM 261100).

Megaloblastic anaemia 1 (MGA1), known as Imerslund–Gräsbeck syndrome, (I-GS) is a rare autosomal recessive condition originally and independently described by Imerslund in Norway (Imerslund 1960) and Gräsbeck and collaborators in Finland (Gräsbeck et al. 1960). At the cellular level the disease is a selective intestinal Vit B12 malabsorption defect that is characterized by the frequent association of juvenile megaloblastic anaemia and a benign proteinuria of the tubular type (Gräsbeck 1972; Wahlstedt-Froberg et al. 2003). Patients have decreased levels of serum Vit B12 in the presence of normal levels of intrinsic factor (IF) (Mackenzie et al. 1972; Burman et al. 1985). Classically, patients present during childhood with pallor, developmental delays and variable neurological symptoms (Campbell et al. 1981; Broch et al. 1984; Salameh et al. 1991; Wulffraat et al. 1994; Altay et al. 1995; Carmel et al. 2003). The therapy consists of periodic parenteral administration of Vit B12 that restores normal metabolism and haematopoiesis.

Currently, more than 200 human MGA1 cases have been reported worldwide, but the disease occurs more frequently in Scandinavian countries: Norway and Finland (Tanner et al. 2004). The disease is also frequent in several Middle Eastern countries, including Turkey, Kuwait, Saudi Arabia and Israel (Ben-Bassat et al. 1969; Yetgin et al. 1983; Abdelaal and Ahmed 1991; Salameh et al. 1991; Altay et al. 1995; Celep et al. 1996; Ismail et al. 1997; Tanner et al. 2004). In Tunisia, 24 cases have been diagnosed, but only some have been published (el Bez et al. 1992; Ben Meriem et al. 1993; Fitouri et al. 2001).

In humans, MGA1 exhibits locus heterogeneity and can be caused by mutations in at least two different genes, cubilin (*CUBN*) (Aminoff et al. 1999) and amnionless (*AMN*) (Tanner et al. 2003). The MGA1 was the first locus to be mapped by linkage analysis in Finnish and Norwegian families to a 6 cM region on chromosome 10p12.1 (Aminoff et al. 1995). A functional candidate gene encoding the intrinsic factor–vitamin B12 (IF-VitB12) receptor, cubilin, has been isolated by positional cloning (Moestrup et al. 1998), and the human gene, *CUBN*, was mapped to the same chromosomal region within the MGA1 locus (Kozyraki et al. 1998). Mutations within the *CUBN* gene have been shown to be the cause of MGA1. This gene encodes for cubilin, a multi-ligand receptor essential for the uptake of Vit B12 in the small intestine (Birn et al. 1997).

In all Finnish families the disease is caused by mutations in the *CUBN* gene, with one founder mutation, P1297L, accounting for the majority of Finnish cases (Aminoff et al. 1999; Tanner et al. 2004).

However, not all I-GS patients have *CUBN* mutations. Through linkage studies of Norwegian and Middle Eastern kindreds (Aminoff et al. 1999; Al-Alami et al. 2002) a second candidate gene, *AMN*, was identified and was located on human chromosome 14q32 (Tanner et al. 2003). Distinct mutations were identified in the *AMN* gene in Norwegian, Jewish, Turkish and Saudi Arabian families (Tanner et al. 2003, 2004). In Norway, all the studied cases were due to mutations in the *AMN* gene, whereas cases from Mediterranean regions had mutations in either the *CUBN* or the *AMN* gene (Tanner et al. 2004). It has been demonstrated that AMN functions in the same pathway as cubilin and forms a complex designated cubam, which is essential for endocytosis/trancytosis of several ligands (Fyfe et al. 2004).

In the present study we report on the first genetic and molecular investigations of MGA1 in a North African population and, particularly, in Tunisian families. For this purpose, linkage analysis and homozygosity mapping were performed in six unrelated consanguineous families, including nine patients. Our data showed evidence for linkage in four families to the *MGA1* locus and in one family to the *AMN* gene. Mutation screening by direct sequencing of all exons of the *AMN* gene allowed the identification of the c.208-2A>G, a mutation recently reported in a Jewish Israeli patient of Tunisian origin. In one multiplex family, haplotype analysis excluded linkage to both *CUBN* and *AMN* genes, suggesting the presence of a third gene locus that may cause MGA1.

Patients and methods

Patients and families

Six unrelated MGA1 families, referred to here as families MA1 to MA6, with a total of nine patients and 19 unaffected family members, were investigated. Consanguinity occurred in all families and was of first or second degree. All patients were female. Three families (MA1, MA5 and MA6) were multiplex, with two affected siblings; two families (MA2 and MA4) were simplex, with one affected and at least one

healthy child; and one singleton family (MA3) with only one affected child. Pedigrees of these families are shown in Fig. 1. Families MA1, MA2, MA3 and MA6 had originated from central Tunisia; two of them (MA1 and MA2) were from the same city; and two families, MA4 and MA5, had originated from cities in northern Tunisia.

Patients were diagnosed on the basis of the association of clinical symptoms, low serum Vit B12 level and normal folate level in vitamins assays assessed by radioimmunoassay methods (Simultrac, SNB–ICN pharmaceuticals). The diagnosis was confirmed by the radiolabelled cobalamin absorption test with and without adjunction of exogenous intrinsic factor (dual-isotope Schilling test, Dicopac test) (Schilling 1953). Patients with MGA1 did not correct their intestinal malabsorption with exogenous IF. The outcome of the disease is always favourable under regular parenteral Vit B12 administration.

Methods

Genotyping

Informed consent was obtained from all families. Genomic DNA was extracted from peripheral blood leucocytes from all patients and unaffected relatives by standard procedures (Sambrook et al. 1989). Microsatellite markers D10S1476, D10S504, CUBN and D10S1714 have been amplified for linkage analysis at 10p12.1. CUBN is an intragenic marker (Aminoff et al. 1999). Microsatellites D14S1006, D14S985, D14S293 and D14S292 localized within the *AMN* gene were employed for the chromosomal segregation study at 14q32, and markers D2S2345, D2S2284 and D4S1614, D4S3023 and D4S394 for linkage analysis to the *LRP2* (megalin) and *LRPAP1* genes (RAP) at chromosomes 2q31.1 and 4p16.3, respectively. The polymorphic markers overlapping each locus tested (interval of

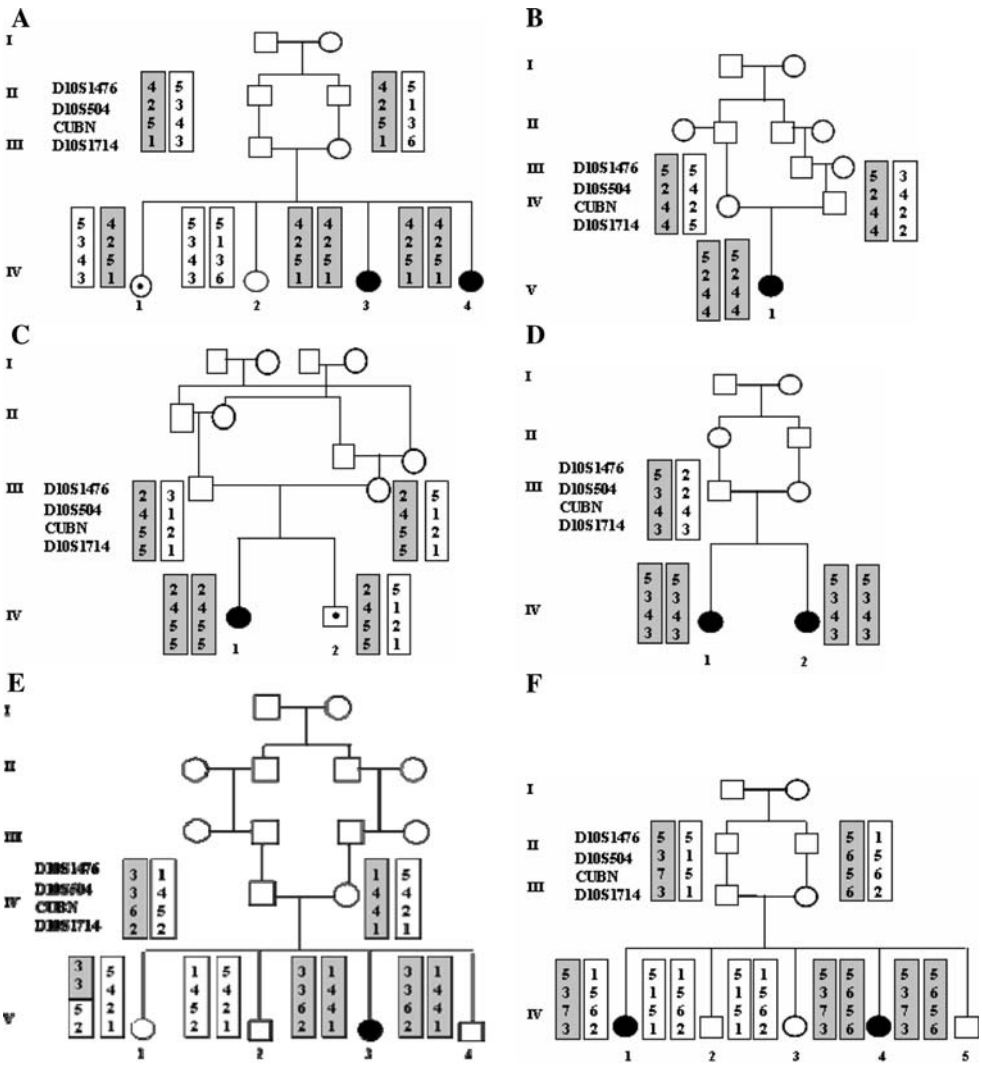

Fig. 1 Pedigrees and haplotype analysis of the six I-GS Tunisian families with four markers in a 4 cM interval overlapping the *CUBN* region. **a**, **b**, **c**, **d**, **e**, **f** represent the families MA1, MA3, MA4, MA5, MA2 and MA6, respectively. *Open squares* and *open circles* represent unaffected males and females. *Filled circles* indicate females with confirmed MGA1. Each generation is designated by a *Roman numeral* (*I–V*). The children genotyped were designated by *Arabic numerals*. The chromosome assumed to carry the disease allele is shown by *shading*

about 2–4 cM (or Mb)) were selected from the Généthon map (Dib et al. 1996). Markers of each polymerase chain reaction (PCR) were amplified in a 50 µl volume by published protocols (Bouchlaka et al. 2003). The PCR amplification products were separated on a 6% polyacrylamide gel and transferred on a Hybond-N^+ membrane (Amersham Biosciences, Amersham, UK) by a contact blotting procedure. Amplified fragments were then revealed by hybridization at 42°C for 3 h in hybridization buffer with a radioactive poly(AC) probe labelled with [α-^{32}P]deoxycytidine triphosphate and visualized by autoradiography (Hazan et al. 1992). Different alleles were arbitrarily numbered, starting with the band of smallest molecular size.

Linkage analysis

Multipoint LOD scores (Log of odds) were computed with Genehunter v2.1 (Kruglyak et al. 1996), and genetic distances were derived from the Généthon map (Dib et al. 1996). LOD scores were calculated with the assumption of a disease allele frequency of 0.0001, fully penetrant autosomal recessive mode of inheritance, and equal marker allele frequencies. Linkage heterogeneity was tested by the admixture test (Smith 1963) implemented in Genehunter, which also estimates the proportion α of families that are linked to the studied locus.

Mutation analysis

Mutation screening was performed by amplification coding regions and intron/exon boundaries with the appropriate primers selected from genomic sequence obtained from databases (http://www.ncbi.nlm.nih.gov). Only some exons of the *CUBN* gene corresponding to the Finnish, Mediterranean and Arab mutations, already described by Tanner and collaborators, (Tanner et al. 2004), and the full coding sequences of the *AMN* and *RAP* genes were analysed. Details of the PCR conditions and primer pairs are available upon request.

PCR products were purified with a QIAquick gel extraction purification kit (Qiagen) and directly sequenced with the Big Dye terminator kit (Applied Biosystems) on an ABI prism 377 DNA sequencer (Applied Biosystems).

Results

Clinical features

In MGA1 the clinical signs are those associated with anaemia, including pallor, fatigue and anorexia, and they tend to be unspecific, rendering the diagnosis difficult. Therefore, there are some criteria that allow the distinction between patients suffering from MGA1 from those with other megaloblastic anaemia. For the nine Tunisian MGA1 patients reported in the present study, the diagnosis of MGA1 was based on the following established criteria (Gräsbeck et al. 1960; Imerslund 1960; Broch et al. 1984): (a) appearance of megaloblastic anaemia within the first 8 years of life (age range 9–84 months), (b2) low serum Vit B12 levels by radioimmunoassay, (c3) normal serum folate level, (d4) no evidence of radiological and endoscopic digestive tract abnormalities, (e5) proteinuria was detected in all cases except one in our series, MA2 patient, (f6) and complete haematological response to regular injections of parenteral Vit B12, (g7) The dual-isotope Schilling test allowed confirmation of MGA1 by revealing intestinal malabsorption of labelled Vit B12, not corrected by the addition of an exogenous intrinsic factor. Clinical features of the nine patients are given in Table 1. In our series, patients did not manifest any neurological symptoms.

Haplotype and linkage analyses

At the start of the present study (January 2002), only mutations in the *CUBN* gene were known as molecular defects underlying I-GS. Therefore, we first investigated the involvement of the *CUBN* gene in nine patients belonging to six unrelated consanguineous families. For this purpose linkage analysis was first undertaken for all families, with four markers covering the *CUBN* gene region (about 2 cM). For each family member, the most likely haplotypes were constructed by visual inspection and by the use of Genehunter, assuming the least number of possible recombinations. Homozygosity mapping showed that, in four families, the patients were homozygous by descent for the four markers tested (Fig. 1). The affected children from each multiplex family (MA1 and MA5) had inherited identical maternal and paternal haplotype. Patients' haplotypes were different from those of the healthy siblings (MA1 and MA3). Segregation of the *CUBN* markers and *CUBN* gene was observed, suggesting that the *CUBN* gene was likely to be responsible for the MGA1 in these families.

For families MA2 and MA6, the linkage to the *CUBN* gene can be excluded, since the affected siblings were not homozygous for the markers tested and the sons who were unaffected clinically had inherited the identical haplotypes as the affected sisters. Furthermore, the two affected children from the multiplex family MA6 did not share the same haplotypes. Mul-

Table 1 Clinical and genealogical data of the nine Tunisian megaloblastic anaemia (MA) patients. ^{58}Co-Cb1(%) indicates the excretion percentage of free labelled Co [^{58}Co], IF-^{57}Co-Cb1(%) indicates the excretion percentage of labelled Co [^{57}Co] bound to intrinsic factor in 24 h urine. *Hb* haemoglobin, *Cb* cobalamin, *Co* cobalt, *MCV* mean cellular volume, *PU* proteinuria, + or – indicates the presence or absence of proteinuria, *NA* not available

Patients	Age at diagnosis (months)	Hb (g/dl)	MCV (fl)	PU	Serum Cb[a] (pmol/l)	Serum folate[b] (nmol/l)	Erythrocyte folate[c] (nmol/l)	Schilling test		Geographic origin	Consanguinity
								^{58}Co-Cb1(%)	IF-^{57}Co-Cb1(%)		
MA1(3)	48	4.3	106	+	35	20	607	0.6	0.1	Central Tunisia	First degree
MA1(4)	18	11.6	126	+	45	14	317	0	0		
MA2(3)	18	5	114	–	NA	NA	NA	0.3	0.1	Central Tunisia	Second degree
MA3(1)	9	4.6	97	+	32.5	61	639	0.1	0.1	Central Tunisia	Second degree
MA4(1)	36	8.2	123	+	88	16	701	0.2	0.3	Northern Tunisia	First degree
MA5(1)	72	4.5	102	+	58	23.3	723	0.7	0.3	Northern Tunisia	First degree
MA5(2)	84	14	108	+	52	92	2675[d]	0.2	1.7		
MA6(1)	48	6	106	+	36	23	723	1	1	Central Tunisia	First degree
MA6(4)	84	8	112	+	22	5.6	343	0.3	1.8		

[a] Normal range was 118–716 pmol/l

[b] Normal range was 3.4–38.4 nmol/l

[c] Normal range was 272–1952 nmol/l

[d] Patient MA5 (2) was under folate treatment

Table 2 Multipoint LOD scores between the MGA1 locus and four markers in six Tunisian MGA1 families

Markers[a]	LOD score	(α, HLOD)
D10S1476	–6.672479	(0.4088, 0.4886)
D10S504	–7.051425	(0.4092, 0.4899)
CUBN	–7.695501	(0.4076, 0.4837)
D10S1714	–4.161658	(0.4241, 0.4967)

[a] Information for all markers was above 95%

Table 3 Multipoint LOD scores between the MGA1 locus and four markers in the MA2 and MA6 families

MA2 family		MA6 family	
Markers	LOD score	Markers	LOD score
D10S1476	–4.173260	D10S1476	–4.075770
D10S504	–3.612118	D10S504	–5.017431
CUBN	–4.544503	CUBN	–4.720697
D10S1714	–1.172476	D10S1714	–4.549077

tipoint LOD scores were computed for all families between the *CUBN* gene and the markers overlapping the *CUBN* region, and heterogeneity was tested by a standard admixture test. Evidence for linkage in the presence of heterogeneity was detected. LOD score in MGA1-linked families varied between 0.12 and 0.85 (data not shown). The estimated proportion of linked family α was 0,41, indicating significant genetic heterogeneity (Table 2). The two families, MA2 and MA6, gave negative LOD scores in this region and provided evidence of their exclusion to the *CUBN* gene (Table 3).

These two families were then typed with four microsatellite markers in a 4 cM region flanking the *AMN* gene. In the MA2 family, haplotype analysis and homozygosity mapping were consistent with linkage to the *AMN* gene, as the affected child was homozygous for all the markers tested and did not share the same parental chromosomes as his unaffected brothers and sister (Fig. 2a). In the multiplex family, MA6, the two affected sisters had inherited different haplotypes, and one of them shared the same chromosomes as her unaffected sister (Fig. 2b). In multipoint linkage tests, the LOD score was under –2.0 throughout the *AMN* region, excluding the implication of the *AMN* locus in this family (Table 4).

Owing to the high affinity between the cubilin and the two proteins RAP and megalin (Birn et al. 1997; Kristiansen et al. 1999; Yammani et al. 2001; Christensen and Birn 2002), we tested the implication of the *LRPAP1* (RAP) or the *LRP2* (megalin) in MGA1 in this latest family. Three microsatellite markers flanking the *LRPAP1* gene and two markers flanking the *LRP2* gene covering regions of 3 and 2 Mb, respectively, were investigated. Linkage to the *LRP2* gene was, however, rejected (data not shown). Analysis with markers of the *LRPAP1* gene showed that the two affected siblings shared the same haplotypes, which were different from those transmitted to the healthy children (Fig. 3). These two affected siblings were

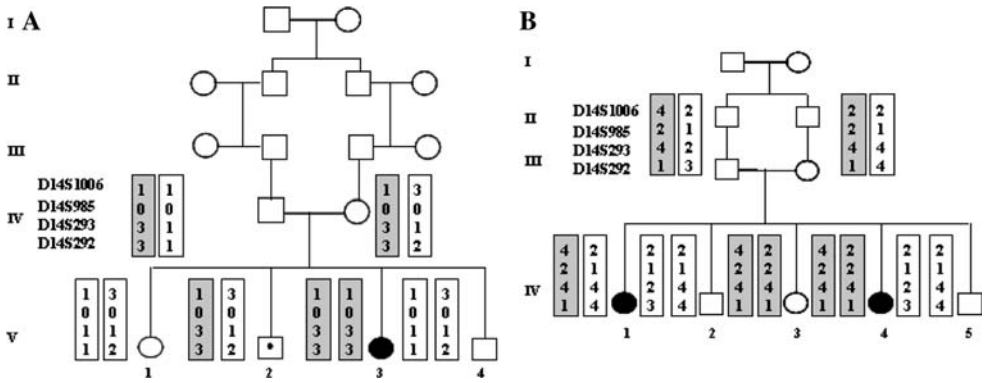

Fig. 2 Pedigrees and haplotype analysis of **a** the MA2 family and **b** the MA6 with markers in a 4 cM region flanking the *AMN* gene region. The *zero* indicates that the alleles were not available. The chromosome assumed to carry the disease allele is shown by *shading*

Table 4 Multipoint LOD scores between the *AMN* gene and four markers in the MA6 family

Markers	LOD score
D14S1006	–3.017376
D14S985	–2.713768
D14S293	–2.698691
D14S292	–3.759653

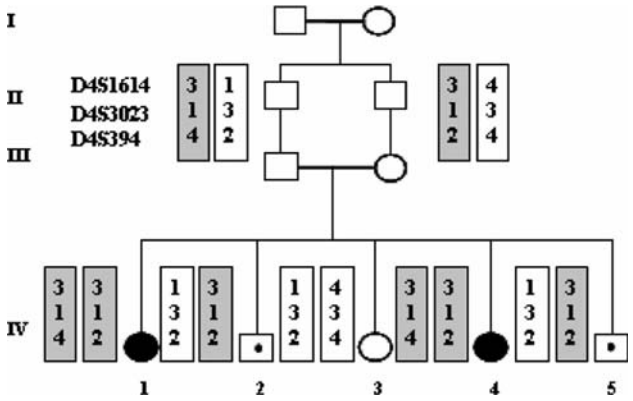

Fig. 3 Pedigree and haplotype analysis of the MA6 family with markers covering a region of 3 Mb within the *LRPAP1* gene corresponding to the RAP protein. The markers are shown from centromeric to telomeric regions of chromosome 4. The haplotype likely to be related to the disease is shown by *shading*

homozygous for the two closest markers flanking the *LRPAP1* gene. On the basis of this result, we cannot exclude the possibility that the disease could be caused by a mutation in the *LRPAP1* gene in this family.

Mutation analysis

MGA1 patients, belonging to four consanguineous families, who were homozygous for *CUBN* haplotypes and, therefore, showed evidence for linkage to the MGA1 locus were analysed for the presence of mutations in the *CUBN* gene. Owing to the large size of the *CUBN* gene, consisting of 67 exons and 66 introns spanning a region of approximately 170 Kb, we partially amplified and sequenced some regions from genomic DNA corresponding to the mutations already described (Aminoff et al. 1999; Tanner et al. 2004). The search for mutations was started by screening for the two *CUBN* Finnish mutations: a missense mutation (FM1, c.3890C>T), which constitutes the major Finnish mutation, and one complex splice mutation (FM2, c.3300-439C>G), occurring in exon 27 and intron 23, respectively. Then, we screened the mutations already identified in the Arabian and Mediterranean families (c.250C>T; c.434G>A and c.1951C>T), found within exons 2, 5 and 16, respectively. None of these mutations or any other mutation in the sequenced regions has been identified among the Tunisian MGA1 patients analysed.

For the patient from the MA2 family, who showed evidence of linkage to the *AMN* gene, the direct sequencing of the full length of the amplified coding region allowed the identification of the c.208-2A>G (Fig. 4). This splice mutation that occurred in intron 3 has been previously described in a Jewish Israeli family of Tunisian origin (Tanner et al. 2003) and in three Turkish families (Tanner et al. 2004) and causes a skipping of exon 4 leading to a subsequent frame shift and premature termination codon in exon 6 (Tanner et al. 2003). This mutation was directly screened for all Tunisian patients, but it was not detected in any of them.

The latest family, MA6, who showed exclusion to both *CUBN* and *AMN* genes and was likely to be linked to the LR*PAP1* gene, was investigated for the presence of mutations in this gene. For this purpose, all exons and the promoter region were amplified and directly sequenced. No mutation was found in the coding region, splice sites or in the 500 bp upstream of exon 1.

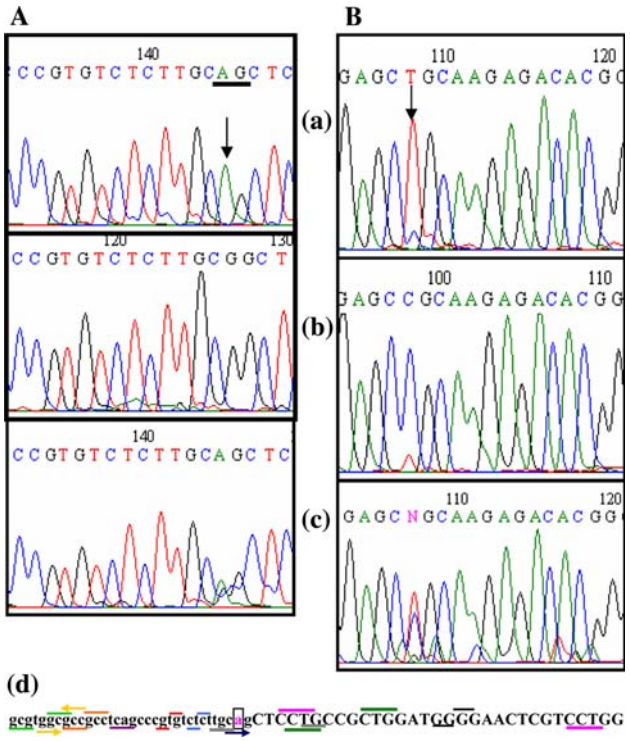

Fig. 4 Sequence chromatograms for the region of the c.208-2A>G splice-site mutation within intron 3 of the *AMN* gene responsible for the MGA1 in the MA2 family. *A* Sense sequence, *B* antisense sequence. **a** Genomic DNA from a wild-type sequence; **b** genomic DNA from a patient that shows the homozygous mutation; **c** genomic DNA from a parent who is a heterozygous carrier for the mutation. *Arrows* indicate mutation position. **d** Analysis of the DNA sequence flanking the c.208-2A>G mutation. The nucleotide substitution (*boxed*) is within a mutation hot spot consensus sequence CCTG and is surrounded by overlapping direct (*underlined*) and inverted repeats (*arrows*). The *lowercase letters* represent a part of intron 3, and the *uppercase letters* represent the first nucleotides of exon 4

Discussion

MGA1 is a relatively rare cause of megaloblastic anaemia due to selective intestinal malabsorption of Vit B12. The current study describes the first molecular and genetic investigation of MGA1 in Tunisian patients. Six unrelated consanguineous families, including nine affected children, were analysed by linkage analysis and homozygosity mapping. All patients fulfilled the criteria of the disease, and the diagnosis was confirmed by the dual-isotope Schilling test. The first step of the study was to test all families with linkage to the MGA1 locus. Genotyping with four markers flanking the *CUBN* gene showed that four families were closely linked to the *CUBN* region, and there was no evidence of an additional implicated gene for these families, although the identification of different haplotypes in this group of patients suggested that more than one common mutation in *CUBN* gene occurred.

These patients were screened for the presence of the already published Finnish, Mediterranean and Arabian *CUBN* mutations by direct sequencing of the corresponding regions from genomic DNA. None of the screened mutation or other mutation in the regions analysed was identified in our population, indicating that these patients may have had mutations in the coding regions not yet analysed, in the introns or in the promoter region.

The two families without evidence of linkage to the *CUBN* gene were then typed with microsatellite markers flanking the *AMN* gene region. Haplotype analysis and homozygosity mapping revealed a linkage to the *AMN* gene in one family (MA2) and rejected the linkage to the other (MA6), indicating that the disorder in the latter family was caused by a gene defect other than *CUBN* and *AMN*.

Sequencing of the full *AMN* coding sequence gene in the patient in the MA2 family allowed the identification of the c.208-2A>G, previously described in a Jewish Israeli patient of Tunisian origin (Tanner et al. 2004). Interestingly, this mutation was also detected in three Turkish families (Tanner et al. 2004). The identification of a common mutation allowed us to address the question of the possible historical and geographic origin of this mutation. On the basis of the known etymological and historical events, it is not surprising to find similar haplotypes in Jewish and non-Jewish families whose origins lie within the Mediterranean basin.

If one considers all the steps involved in the transport of Vit B12 across the enterocytes, and due to the structure of the cubilin and its ability to bind to multiple ligands, it is likely that another not yet identified gene might be involved in the intestinal malabsorption of Vit B12 that caused MGA1 in the MA6 family.

Because cubilin binds to megalin and RAP with high affinity and co-localizes in several tissues, it is highly conceivable that mutations in one of the two genes can be the cause of MGA1 in this family. For this purpose, we tested the possibility of linkage of the disease locus in this family to the two genes. Homozygosity mapping and haplotype analysis excluded a linkage to megalin but could not exclude a linkage to RAP. Sequencing of the entire *LRPAP1* coding region, as well as the promoter region, revealed no abnormalities in any of the coding sequences and intron/exon junctions. For this family, it seems likely that a mutation in the regulatory regions of the RAP gene could be the underlying defect in these patients. The other possibility is that the disease in this family may result in the alteration of a

third MGA1 gene that has not yet been identified and causes a similar phenotype.

A recent study has demonstrated that the human gene encoding the gastric intrinsic factor (GIF) is mutated in some patients for whom linkage to both *CUBN* and *AMN* has been excluded. These patients had been diagnosed initially as having I-GS, but it was then concluded that they were suffering from intrinsic factor deficiency (Tanner et al. 2005). Involvement of this gene in the MA6 family is unlikely, because the Schilling test carried out on the two affected sisters revealed intestinal malabsorption of labelled Vit B12, which was not corrected by the addition of an exogenous intrinsic factor.

The present study demonstrates that, in spite of the high rate of endogamous marriages in Tunisia, there was a genetic and a mutational heterogeneity affecting the Tunisian MGA1 patients, confirming previous data on several Mendelian diseases affecting the Tunisian population (Elloumi-Zghal et al. 2002; Bouchlaka et al. 2003, Charfeddine et al. 2003). The characterization of new genes and new mutations responsible for MGA1 will, in the future, yield new biological insights and a better comprehension of all the steps involved in the transport of Vit B12 and also facilitate a more exact diagnosis of new suspected cases at an earlier stage of the disease, which is important for the appropriate treatment.

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