Localization of the Gene for Thiamine-Responsive Megaloblastic Anemia Syndrome, on the Long Arm of Chromosome 1, by Homozygosity Mapping

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Summary

Thiamine-responsive megaloblastic anemia, also known as "TRMA" or "Rogers syndrome," is an early-onset autosomal recessive disorder defined by the occurrence of megaloblastic anemia, diabetes mellitus, and sensorineural deafness, responding in varying degrees to thiamine treatment. On the basis of a linkage analysis of affected families of Alaskan and of Italian origin, we found, using homozygosity mapping, that the TRMA-syndrome gene maps to a region on chromosome 1q23.2-23.3 (maximum LOD score of 3.7 for D1S1679). By use of additional consanguineous kindreds of Israeli-Arab origin, the putative disease-gene interval also has been confirmed and narrowed, suggesting genetic homogeneity. Linkage analysis generated the highest combined LOD-score value, 8.1 at a recombination fraction of 0, with marker D1S2799. Haplotype analysis and recombination events narrowed the TRMA locus to a 16-cM region between markers D1S194 and D1S2786. Several heterozygote parents had diabetes mellitus, deafness, or megaloblastic anemia, which raised the possibility that mutations at this locus predispose carriers in general to these manifestations. Characterization of the metabolic defect of TRMA may shed light on the role of thiamine deficiency in such common diseases.

Introduction

Thiamine-responsive megaloblastic anemia (TRMA; OMIM 249270 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?249270]), first described by Rogers et al. (1969), is an autosomal recessive disorder with childhood onset. To date, clinical data from ~12 families from various ethnic groups have been published, and several other kindreds are known (Rogers et al. 1969; Cagianut et al. 1977; Viana and Carvalho 1978; La Grutta et al. 1980; Haworth et al. 1982; Mandel et al. 1984; Abboud et al. 1985; Rosskamp et al. 1985; Grill et al. 1991; Morimoto et al. 1992; Akinci et al. 1993; Rindi et al. 1994; Schorderet et al. 1994). Most of the TRMA patients, both boys and girls, originated from consanguineous families or from couples whose families had lived for many years in the same small village, which is consistent with autosomal recessive inheritance. The cardinal clinical manifestations of the syndrome are megaloblastic anemia, diabetes mellitus, and sensorineural deafness, all of which respond in varying degrees to the administration of thiamine (vitamin B1), in pharmacological doses. The diabetes, which appears in childhood, is a novel category of diabetes mellitus and is non–type I in nature: in some cases, the insulin requirement is reduced during thiamine therapy (Borgna-Pignatti et al. 1989*a*; Rindi et al. 1992; Mandel et al. 1993). Anti-insulin and anti–islet cell antibodies are absent in patients (Borgna-Pignatti et al. 1989*a*; Mandel et al. 1993; Rindi et al. 1994). In addition to the cardinal findings for which the syndrome is named, some patients show congenital heart disease and/or arrythmias (Viana and Carvalho 1978; Mandel et al. 1984; Abboud et al. 1985; Poggi et al. 1989), as well as abnormalities of the retina and the optic nerve (Mandel et al. 1984; Borgna-Pignatti et al. 1989*a*; Rindi et al. 1994).

The pathophysiology of TRMA remains obscure but is of considerable interest. In the present study, we have
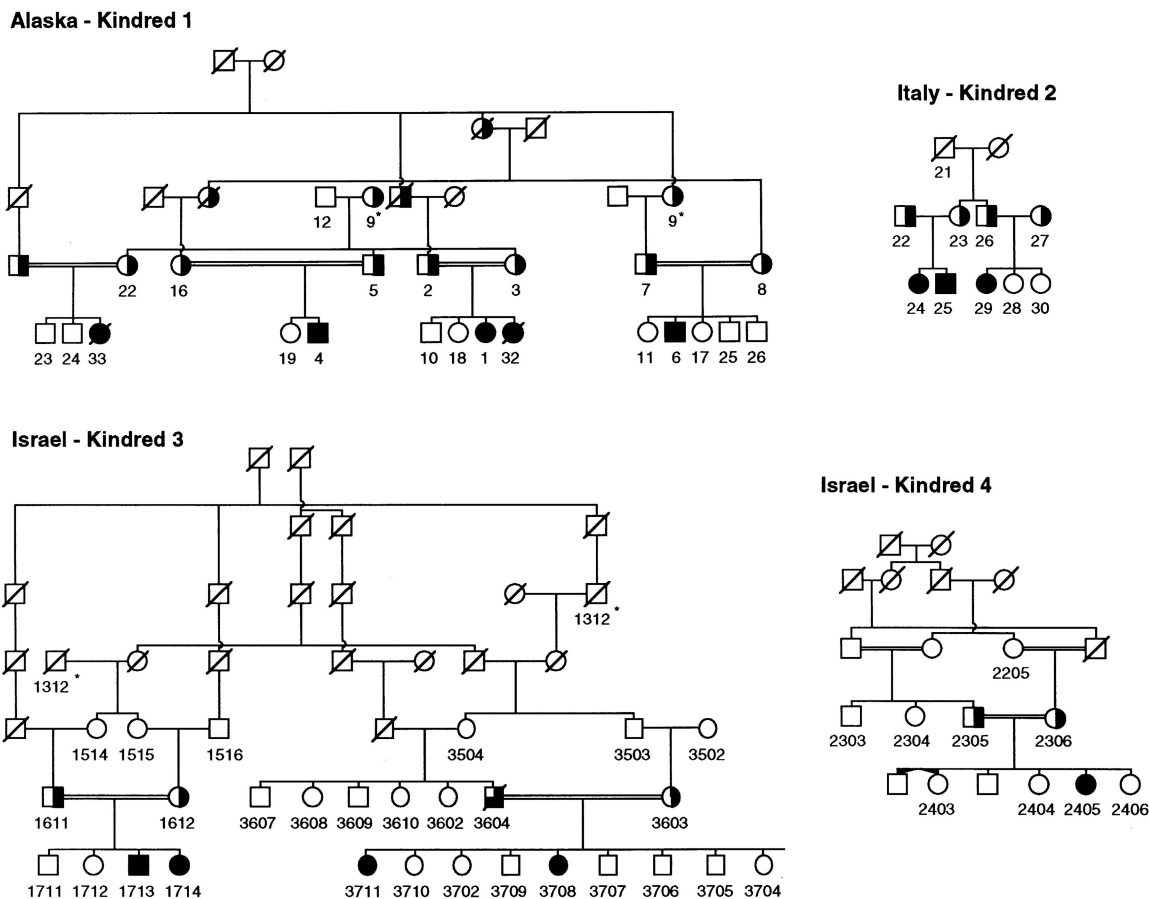
Figure 1 Pedigrees of the four TRMA kindreds. Kindreds 1, 3, and 4 are consanguineous. The symbol used for individual 3604 (kindred 3) indicates that, in addition to being an obligate heterozygote, this individual had diabetes and deafness. Blood samples and genotypes were obtained for all numbered individuals (except 3604 [kindred 3] and 33 and 32 [kindred 1]). For clarity, subjects 9 (kindred 1) and 1312 (kindred 3) are represented twice, because they had multiple mates. Altogether, we had access to 11 patients and 56 healthy subjects.

used the homozygosity-mapping strategy (Lander and Botstein 1987) to localize the TRMA gene to the long arm of chromosome 1 (band 1q23.2-23.3), using families of Alaskan, of Italian, and of Israeli-Arab origin. This will serve as a first step toward the identification of a gene that is important in thiamine metabolism and that plays a role in the pathophysiology of diabetes, deafness, and anemia.

Subjects and Methods

Families and DNA Preparation

Four unrelated kindreds of various ethnic origin, three of which were consanguineous, were included in the study. The family trees of all the kindreds are shown in figure 1. Kindred 1 is a consanguineous family living in a native village in coastal Alaska (E. J. Neufeld and N. Buist, unpublished data). The ethnic background of the villagers is part native Alaskan and part Russian

European. Kindred 2 is of Italian origin and initially was reported as having thiamine-responsive anemia with Wolfram syndrome, also known as "DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy, and deafness) syndrome" (OMIM 223300 [http://www.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?223300]) (Borgna-Pignatti et al. 1989a). However, the fact that additional DIDMOAD-syndrome patients did not have anemia and did not respond to thiamine (Borgna-Pignatti et al. 1989b; Schwingshandl and Borkenstein 1989) contradicted this hypothesis and suggested that the two patients had TRMA and not DIDMOAD syndrome. For this pedigree, the parents were not known to be related; however, the families had lived on the same Venetian island for centuries. Kindreds 3 and 4 are two unrelated Israeli-Arab consanguineous families (Mandel et al. 1984, 1993; Rindi et al. 1994). These two families live in two close villages in northern Israel. All the TRMA patients involved in this study had

the triad of megaloblastic anemia, diabetes mellitus, and deafness during the first years of life and responded in varying degrees to thiamine treatment (Mandel et al. 1984; Borgna-Pignatti et al. 1989*a*; Mandel et al. 1993; Rindi et al. 1994). Blood samples for genetic analysis were collected after informed consent was obtained from the subjects or their guardian, in accordance with the guidelines of local institutions. DNA was prepared by use of standard methods (Sambrook et al. 1989).

Genotype Analysis

Genomewide screening was performed on an automatic sequencing machine using a panel of 374 microsatellite markers spaced at ~10-cM intervals. The marker set closely approximates Marshfield screening set 8 (for a description, see http://www.marshmed.org/genetics/). To construct extended haplotypes, genotyping was performed by use of a nonradioactive-labeling procedure with the ECL system (Amersham), on the basis of a horseradish peroxidase–mediated chemiluminescent reaction, in accordance with the study by Vignal et al. (1993).

Statistical Analysis

Two-point linkage analysis of the disease locus and the genetic markers was performed by use of the MLINK program from the FASTLINK optimized version of the LINKAGE package (Lathrop et al. 1984). Owing to the extreme inbreeding in the TRMA Alaskan family (kindred 1) and Israeli-Arab family 3, which contain four and six loops of consanguinity, respectively, special attention was paid to the pedigree members declared to be so-called loop breakers. The Italian family was considered to be nonconsanguineous and was analyzed in this fashion with MLINK. An autosomal recessive model with complete penetrance in both sexes and a frequency of .001 for the disease allele was assumed. Since the allele frequencies of the markers were not known for the particular population groups of the study, we estimated population allele frequencies from the data. In test runs of MLINK, we found this to be the most conservative estimation of LOD scores (compared with results under the assumption of equal allele frequencies). A sex-averaged genetic map (Dib et al. 1996) was obtained for the markers that were used in haplotype analysis (fig. 2).

Results

Clinical Manifestations in TRMA Heterozygous Carriers

None of the obligate heterozygous parents presented at childhood with the three cardinal features of the disease. However, in kindred 1, individual 7 had glucose intolerance, treated through diet. Severe deafness had been reported in three family members of kindred 2 (Borgna-Pignatti et al. 1989*a*). A common grandfather (individual 21 in kindred 2) had adult-onset diabetes mellitus and a hearing defect that required a hearing aid. In kindred 3, individual 3604, who is the father of two TRMA siblings, had an abnormal glucose-tolerance test, high-tone hearing loss, and macrocytic anemia (Hg 10.2 g/dl) with a mean corpuscular volume of 102, at age 42 years. He died at age 45 years, during an acute asthmatic attack.

Primary Mapping of the TRMA Locus in the Alaskan and Italian Kindreds

A genomewide screen of the TRMA-affected kindreds of Alaskan and of Italian origin (kindreds 1 and 2, respectively; fig. 1) was performed. LOD scores were calculated for each marker, which enabled us to exclude up to 80% of the genome with LOD scores $\leqslant -2$. In the initial screen, the only marker (D1S1679) with a LOD score >3.0 was found on chromosome 1q. Nearby markers (D1S1653 and D1S2141) also gave positive LOD scores, suggesting linkage to the disease within these families. This finding suggested that the TRMA locus maps to chromosome 1q at least in the Alaskan and Italian families.

Confirmation of Linkage of the TRMA Locus to 1q Markers in the Israeli-Arab Kindreds

The next step was to check whether Israeli-Arab kindred 3 was positively linked to the same markers (D1S1679, D1S1653, and D1S2141). For this family, positive LOD scores were obtained as well with these markers, with marker D1S1679 giving the highest combined LOD-score value. There was a broad maximum likelihood (LOD > 4 at a recombination fraction of .03–.12) when data from kindreds 1–3 were combined. Kindred 3 was not fully informative at this locus, but two recombination events were noted in the informative sibship.

Linkage then was verified in the Alaskan (kindred 1) and Israeli-Arab (kindreds 3 and 4) families, by use of 17 microsatellite markers from the Généthon microsatellite panel covering the same chromosomal region (Dib et al. 1996). These markers were telomeric to markers D1S1679, D1S1653, and D1S2141, on the basis of the location database (http://cedar.genetics.soton.ac.uk/pub/chrom1/map.html). Two-point linkage analysis was performed (Lathrop et al. 1984). Evidence for linkage to this region was found in all kindreds tested. Positive LOD scores were obtained for each marker. Marker D1S2799 gave the highest combined LOD-score value, 8.1 at $\theta = 0$, for the three families (1, 3, and 4). These data confirmed linkage of the TRMA locus to chromosome 1q and suggested genetic homogeneity.
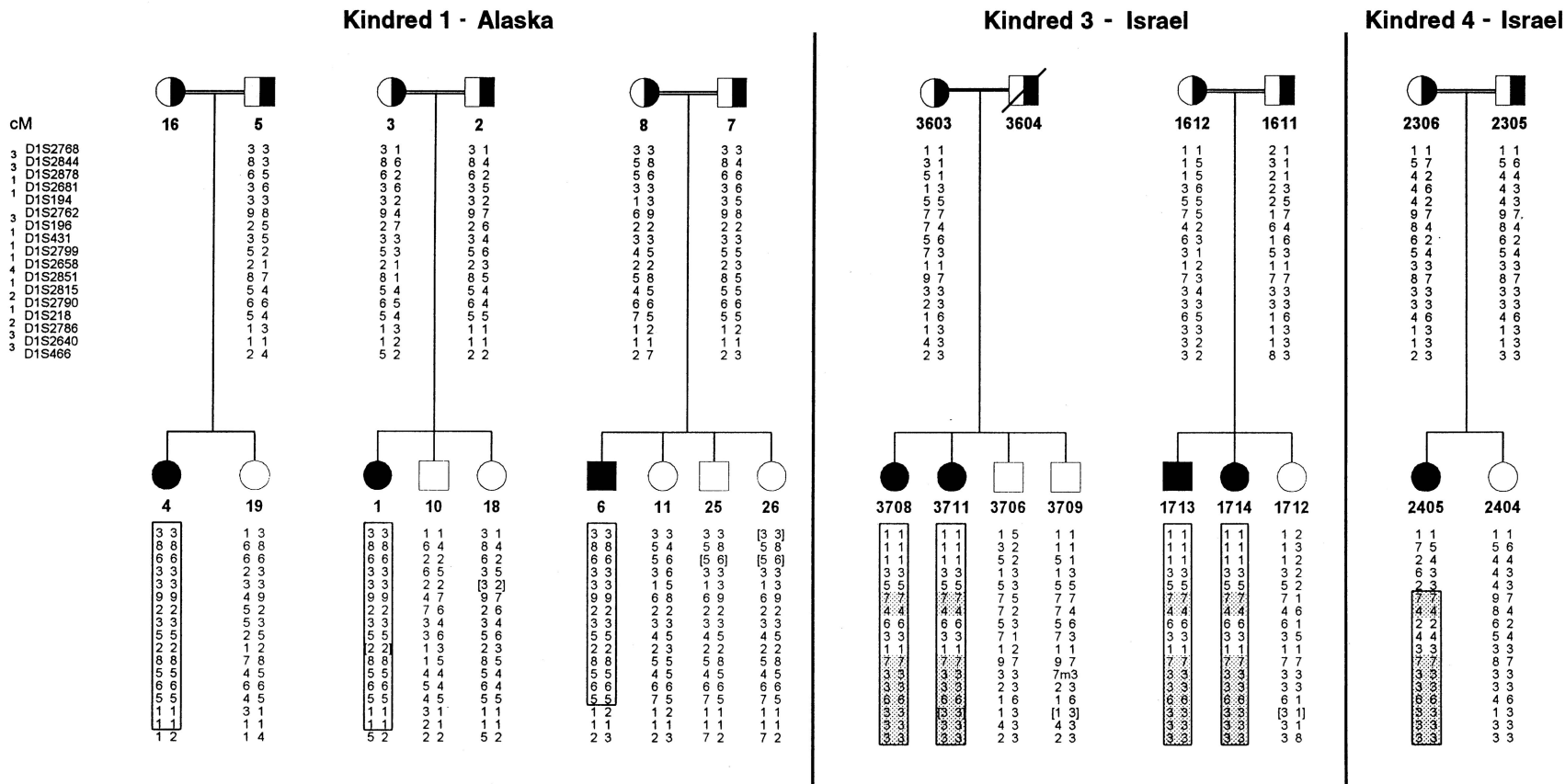
Figure 2 Haplotype analysis of chromosome 1q.23.2-23.3 for TRMA kindreds 1, 3, and 4. Only key subjects are shown. Centromeric to telomeric markers are arranged from top to bottom. The linked haplotype is boxed. Shaded areas correspond to segments of haplotypes shared among the Israeli-Arab patients. A mutation or a scoring failure (indicated with an "m") is seen in subject 3709 (kindred 3). The alleles within brackets are inferred. Genotyping in subject 16 (kindred 1) was technically unsatisfactory and, therefore, is not shown.

Haplotype Analysis and Recombination Events in the Consanguineous Kindreds

Extended haplotypes were constructed, by use of the 17 microsatellite markers, for all subjects from the consanguineous kindreds (1, 3, and 4). Results for each informative individual are shown in figure 2. As expected on the basis of homozygosity by descent, regions of homozygosity were observed in the genomes of all affected individuals. Each of the eight subjects with TRMA was homozygous for all loci between and including D1S2762 and D1S218. Affected individual 2405 (Israeli-Arab kindred 4) was heterozygous at marker D1S194, owing to a recombination event in the paternal haplotype. This enabled us to define the centromeric limit of the interval where the TRMA disease gene lies, at D1S194. As a result of recombination events in both the maternal and paternal haplotypes, affected individual 6 (kindred 1) was heterozygous at marker D1S2786, enabling us to define the telomeric limit of the guilty interval, at this marker. Thus, we primarily defined the TRMA gene interval to a region of 16 cM, extending between but excluding markers D1S194 and D1S2786, by homozygosity mapping. As shown in figure 2, the disease-associated haplotype in the Alaskan patients (kindred 1) bore no relation to the affected chromosome in the Israeli-Arab patients (kindreds 3 and 4). However, haplotypes in the Israeli-Arab patients from the two subfamilies of kindred 3 (patients 3711, 3708, 1713, and 1714) were identical. Comparison of this set of haplotypes with haplotypes from patient 2405 (kindred 4) showed two segments of shared haplotype (D1S2762–D1S196 and D1S2851–D1S466) and two segments where the haplotypes differed (D1S2768–D1S194 and D1S431–D1S2658).

Discussion

This is the first linkage study of TRMA syndrome. The gene for TRMA syndrome is located within a 16-cM region on chromosome 1q23.2-23.3. The present data suggest genetic homogeneity for the disease. This study emphasizes the power of a dense map of microsatellite markers, combined with homozygosity mapping, to map rare recessive disease-causing genes when genetically isolated populations, consisting of large consanguineous families, are available.

Three kinds of disease-associated haplotypes were observed, suggesting the existence of different mutations causing the disease in each family, with a founder effect for each of the mutations. Alternatively, a single disease-gene mutation might have arisen separately within each family and might be the cause of the disease. Haplotype analysis of additional TRMA families reported in the literature may uncover linkage disequilibrium between markers and the disease locus and may narrow the critical interval (Hastbacka et al. 1992; Sirugo et al. 1992). The closely related haplotypes in Israeli-Arab kindreds 3 and 4 may represent this type of linkage disequilibrium.

It has been demonstrated recently that TRMA is consistently associated with a subtle defect in thiamine transport across cellular membranes and with impaired intracellular pyrophosphorylation (Rindi et al. 1994). These studies suggest that the TRMA syndrome represents a novel biochemical defect in thiamine metabolism. A nuclear-gene defect in the thiamine-transport mechanism or in phosphorylation, causing thiamine deficiency, could be the cause of the disease. The metabolic defect in TRMA leads to a multisystem disorder. Interestingly, similar clinical features are found in the more common, autosomal recessive Wolfram syndrome (Borgna-Pignatti et al. 1989a). Recently, a Wolfram-syndrome gene has been linked to markers on chromosome 4p16 (Polymeropoulos et al. 1994), excluding the possibility that these two disorders are caused by defects in the same gene. Another study proposed that a nuclear-encoded defect in Wolfram syndrome is the inability to repair mtDNA defects (Barrientos et al. 1996). Such a mechanism also could underlie the pleiotropic features of TRMA.

Numerous cases of mutations in genes, such as the ataxia-telangiectasia gene (Swift et al. 1991; Athma et al. 1996), that cause both recessive and dominant diseases with more or less severe phenotypes of the same disease have now been reported. In the present study, as well as in other reports (Viana and Carvalho 1978; Haworth et al. 1982; Mandel et al. 1984; Borgna-Pignatti et al. 1989a; Poggi et al. 1989), several obligatory carriers and other family members exhibited some of the clinical manifestations of TRMA, including diabetes mellitus, deafness, or megaloblastic anemia. These findings suggest the predisposition of TRMA heterozygotes to these disorders and raise the possibility that this gene defect could, with other genes, contribute to the heterogeneity of diabetes mellitus, deafness, and megaloblastic anemia.

In conclusion, the interval defined so far, which contains the TRMA gene, lies on chromosome 1q23.2-23.3, in a region with no obvious candidate gene but in close proximity to the genes for coagulation factor V, the selectins, and FAS ligand (183–197 cM in the UniGene map [http://www.ncbi.nlm.nih.gov/UniGene/]). Radiation hybrid maps suggest that there are at least 100 genes that lie in this region, on the basis of the UniGene map. The syntenic portion of murine chromosome 1 (82–90 cM) contains no known genes for autosomal recessive deafness, diabetes, or anemia. Thus, it seems probable that a combination of positional cloning and candidate-gene methods will be required for the identification of

the TRMA gene itself. The genetic homogeneity observed thus far, if confirmed in other families from different ethnic backgrounds, will facilitate the fine mapping of this locus. Analysis of additional families is now underway. In view of the multisystem manifestations of TRMA, it is conceivable that the identification of the molecular basis of TRMA may provide new insights into the role of thiamine in common diseases in the general population, such as type II diabetes mellitus, deafness, and inherited and acquired bone-marrow disorders. It is also conceivable that some patients without the triad of anemia, deafness, and diabetes will have defects in the TRMA gene. One immediate benefit of the mapping effort to date is the potential for carrier screening and prenatal diagnosis, by linkage of TRMA to microsatellite-repeat markers spanning the region. This would enable thiamine administration during pregnancy, which could ameliorate the serious complications of TRMA.

Acknowledgments

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