

## Short reports

## Evidence for exclusion of a mutation in NRAMP as the cause of familial disseminated atypical mycobacterial infection in a Maltese kindred

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## Abstract

In mice, susceptibility to intracellular infections in inbred strains is controlled by a single locus, *Lsh/ItylBcg*, and the gene responsible has been cloned and designated *Nramp* (Natural resistance associated macrophage protein). We have identified a group of related children who appear to have a single gene defect, inherited recessively, which results in increased susceptibility to mycobacterial infection. The immunological defect observed in the affected children resembles that in mice homozygous for the *Lsh/ItylBcg* susceptible allele. To test the hy-

pothesis that a mutation in NRAMP is responsible for the immunodeficiency observed in the affected children, we have typed eight markers in the region of human 2q34-q37 where NRAMP, the human homologue of *Nramp*, maps. We have shown discordance with the defect in one family and the chromosomes in the three affected children have different haplotypes making it unlikely that inheritance of an ancestral mutation in the NRAMP gene is the cause of increased mycobacterial susceptibility in this group of children.

(J Med Genet 1995;32:904-906)

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Received 6 April 1995  
Revised version accepted for publication 9 June 1995

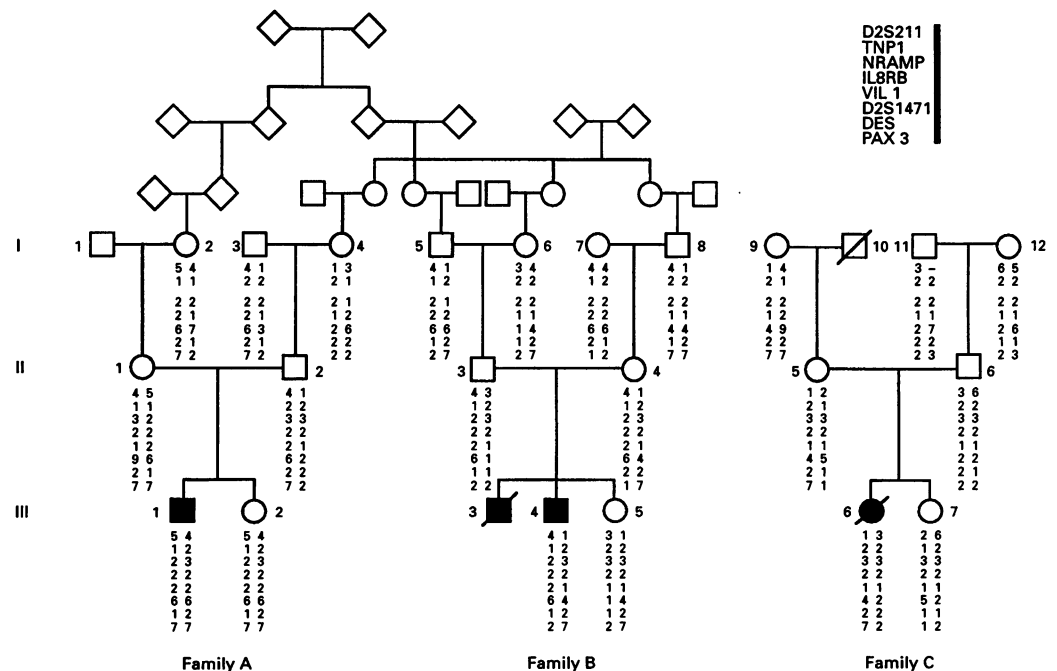


Figure 1 Partial pedigree of the Maltese kindred showing segregation of alleles at eight loci, D2S211, TNP1, NRAMP, IL8RB, VIL1, D2S1471, DES, and PAX3. Affected subjects are represented by closed symbols. Alleles are shown beneath each person genotyped. VIL1 is a 600 bp BamHII/HindIII cDNA probe encoding the 3' region of the villin gene which detects a biallelic MspI polymorphism.<sup>2</sup> IL8RB is a 1.5 kb cDNA probe that detects a biallelic DraI polymorphism.<sup>3</sup> TNP1 is an MspI RFLP within a 1.7 kb fragment of the human transition protein 1 (TNP1) gene which was amplified by PCR before digestion.<sup>4</sup> DES is an EcoRV RFLP within a fragment of the desmin gene<sup>5</sup> which was amplified with the primers 5'GCATGAAGAGGTATACCTGGCC3' and 5'GATGGCCAAAGGTCACAAAAGT3' before digestion. D2S211,<sup>5</sup> PAX3,<sup>6</sup> D2S1471,<sup>7</sup> and NRAMP<sup>8</sup> are dinucleotide repeats which were amplified by PCR.

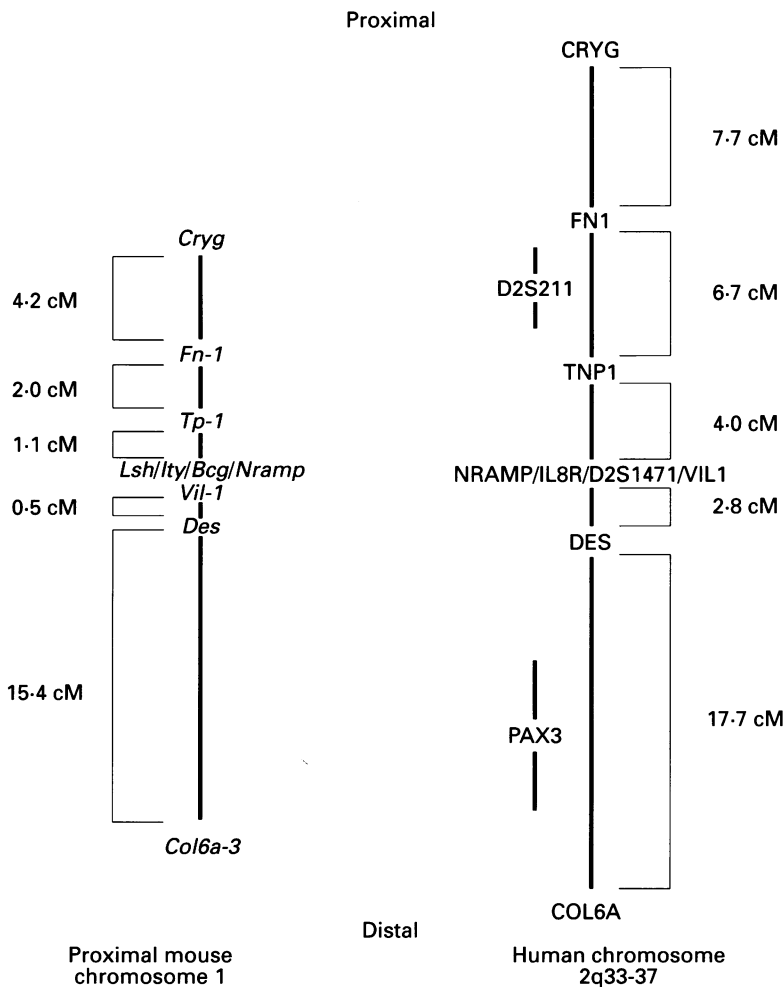


Figure 2 Schematic representation of the syntenic regions of proximal mouse chromosome 1<sup>11</sup> and human chromosome 2q33-37.<sup>3,12</sup> Distances are indicated in centimorgans.

Four children from a village in Malta have presented with disseminated atypical mycobacterial infection. This is usually associated with underlying immunosuppression, whether inherited or acquired, and identification of the defect in this group of children would lead to a better understanding of the pathways involved in the control of infection by intracellular pathogens such as *Mycobacterium tuberculosis*, the cause of tuberculosis.

The clinical features are described in detail elsewhere.<sup>1</sup> Each child is infected with a different bacterial species indicating an underlying defect in host immunity rather than exposure to an unusually virulent organism. However, extensive investigation has excluded all known immune defects usually associated with mycobacterial infection.<sup>1</sup>

The families are shown in fig 1. In the large pedigree, two affected sibs are from a second cousin marriage (family B) while the third affected (family A) is related to the first two as a fourth cousin on both parents' sides. The exact relationship linking the fourth affected child to the large pedigree has not been determined, but she comes from the same relatively isolated village. The presence of two affected sibs in a consanguineous marriage is indicative of autosomal recessive inheritance. Owing to the high degree of inbreeding and the rarity of the disease, it is likely that all four

children have the same autosomal recessive disorder inherited from a common ancestor.

In mice, a recessive mutation in a single gene denoted *Lsh*, *Ity*, or *Bcg* causes susceptibility to intracellular pathogens such as leishmania, salmonella, and mycobacteria.<sup>9</sup> Macrophages from homozygous mutant mice show a variety of specific defects in tumoricidal and antimicrobial activity.<sup>9</sup> The affected children have increased susceptibility to mycobacterial infection and abnormalities of macrophage priming and activation that are very similar to those observed in susceptible mice.<sup>1</sup>

A candidate for the *Lsh/Ity/Bcg* gene has been cloned and designated natural resistance associated macrophage protein (*Nramp*). *Nramp* is located on the proximal region of mouse chromosome 1<sup>10</sup> which is syntenic to human chromosome 2q, as shown in fig 2. In the mouse, *Nramp* is located between *Vil* and *Tp-1*, about 50 kb from *Vil*.<sup>10</sup> Conserved synteny places the human NRAMP gene between markers D2S1471/VIL1 and markers TNP1/D2S211. This is confirmed by physical mapping studies which also show that the human NRAMP gene is located approximately 155 kb proximal to VIL1 on chromosome 2q35.<sup>3,8</sup>

DNA from three affected children and their parents and grandparents were typed with eight polymorphic loci, the positions of which are shown in fig 2. As can be seen in fig 1 all the families show mendelian inheritance of the markers and no recombination events have occurred.

If a mutation in the human NRAMP gene were responsible for the immunodeficiency seen in this Maltese kindred, and the mutation occurred in a common ancestor, then the affected children would share a haplotype for the locus and would be homozygous for it.<sup>13</sup> However, it can be seen that the six chromosomes carry five different haplotypes. In addition, the two sibs in family A, III·1 (who is affected) and III·2 (who is not affected), have inherited the same genotype from their parents. This excludes a recessive mutation in NRAMP unless III·2 has the mutation but has not shown the phenotype. This seems unlikely as she is 6 years old, three times the age of onset of the affected children.

If the children have inherited a recessive mutation, these data indicate that the mutation is unlikely to be in the human NRAMP gene.

M Newport is supported by the St Mary's Hospital Medical School Special Trustees. Work in the Cambridge laboratory is supported by the Wellcome Trust. We thank Dr John Todd for making available the primer sequences for the microsatellite D2S1471.

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