*correspondence*

# Nemaline myopathy mechanisms

Sir — The report by Laing *et al.*[1] in *Nature Genetics* demonstrates that a point mutation in the α-tropomyosin gene (*TPM3*) is responsible for nemaline myopathy. Not mentioned in the paper are two observations that may be relevant to the understanding of this disease.

First, the mutated transcript of *TPM3* is expressed both in cardiac and skeletal muscles. This gene generates two mRNAs by alternative splicing. The 2.5 kb mRNA encodes a non-muscle tropomyosin and is expressed in fibroblasts[2]. This mRNA is present in the human genome as eight mRNA-derived pseudogenes[3,4]. It is interesting to note that this is the tropomyosin mRNA that was found fused to a tyrosine kinase, domain in the *trk* oncogene[2,5]. The 2.5 kb transcript does not contain the exon $I_{sk}$ where the mutation was mapped. The second mRNA is 1.3 kb long and encodes a 284-amino acid skeletal

muscle tropomyosin containing exon $I_{sk}$[2]. This mRNA is expressed both in human skeletal and cardiac muscles[2] and is not present as a pseudogene in the human genome[4]. The presence of the 1.3 kb transcript containing exon $I_{sk}$ in cardiac muscle raises the possibility that the mutated protein may have an effect in the heart.

Second, the mutated $Met_9$ residue occupies an 'a' position in the tropomyosin heptad repeat. Tropomyosins are dimeric proteins composed of two α-helical subunits organized in a coiled-coil structure. Coiled-coil were predicted by Francis Crick in 1953 and found for the first time in tropomyosin[7]. The sequence 'signature' of a coiled-coil protein is the presence of a series of consecutive seven-residue repeats. In these repeats, the first ('a') and fourth positions ('d') are always occupied by hydrophobic amino-acids. These residues form a long hydrophobic

interface between the two α-helices stabilizing the dimer. Leucine zippers are particular cases where leucines are found in the 'a' and 'd' positions. As pointed out by Laing *et al.*[1], the mutation found in the N-terminal region of tropomyosin replaces a highly conserved Met residue for an Arg residue. It should be noted that this Arg residue occupies an 'a' position in the heptad repeat, a 'forbidden' position for charged residues. These positions are never occupied by a charged residue in any of the known skeletal muscle tropomyosins. This might be responsible in part for the observed phenotype.

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# Linkage findings in bipolar disorder

Sir — Straub *et al.*[1] recently described results of linkage analysis between bipolar affective disorder and markers on chromosome 21q22.3, of which *PFKL* and *D21S171* were tested in 47 of their pedigrees. In an affecteds-only analysis, one family yielded a lod of 3.41 (θ=0.0). In a three-point analysis of all families with *PFKL* and *D21S171*, a maximum lod of 2.8 was produced at about 30 centiMorgans (cM) on the *D21S171* side.

We have carried out linkage analysis using *PFKL*, *D21S171* and *D21S49* in six Icelandic and 17 English pedigrees multiply affected with bipolar and unipolar affective disorder, and selected for demonstrating unilineal transmission of bipolar disorder. The transmission models used and five of the Icelandic pedigrees have been described elsewhere[2]. Two hierarchical models of affection were used, denoted BP and UP. The BP model included as affected all those with RDC diagnoses of bipolar I,

bipolar II, mania or hypomania. The UP model included as affected all those with any bipolar or unipolar affective illness except for unipolar depression combined with alcohol abuse.

DNA was obtained from 278 of the 510 subjects in the 23 pedigrees. 97 subjects were affected according to the BP model, and an additional 82 were affected according to the UP model. Linkage analysis was carried out with MLINK and LINKMAP using two affection models with a dominant mode of transmission and in the context of presumed locus heterogeneity. For the first model, only BP cases were counted as affected and the penetrance was set to 0.4 with a normal homozygote penetrance of 0.01 and a gene frequency of 0.006. For the second model the UP cases were additionally included as affected with separate penetrance values of 0.4 and 0.04, yielding overall penetrances for affective disorder of 0.8

and 0.05 for heterozygotes and normal homozygotes respectively. In each model all subjects not included as affected were classified as unaffected. These affection models and transmission model parameters were chosen to maintain consistency with our previous investigations[2], and do not correspond exactly with the diagnostic model used by Straub *et al.*[1]. In addition to lod score analysis an identity-by-descent (IBD) sib pair analysis was performed for each model. The markers were genotyped as by Straub *et al.*[1], and the allele frequencies were as published in the Genome Data Base (GDB), except that rare extra alleles were detected at *D21S49* and *D21S171* and these were assigned population frequencies of 0.01.

Using the BP-only affection model, the maximum two-point lod of 1.28 was obtained with *D21S171* (θ=0.01, α=0.35). Three Icelandic families produced positive lods at θ=0 with