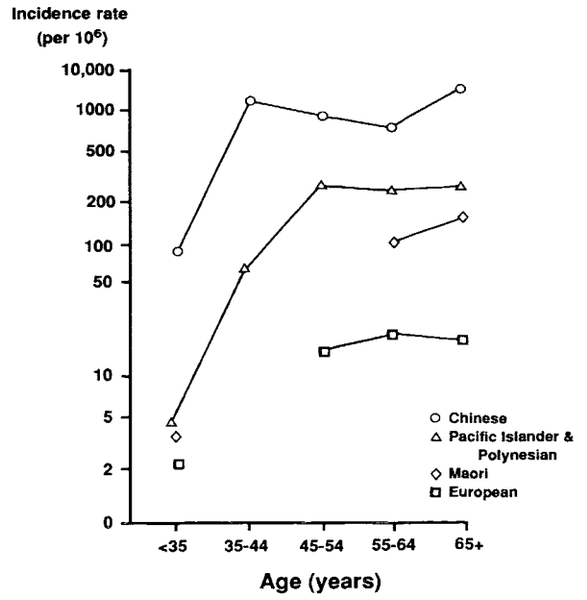

Incidence of NPC 1976–85: Auckland, NZ, men.

adjusted incidence rate for NPC intermediate between the rates for Europeans and Chinese.

Using data from the Auckland regional cancer registry for 1976–85 and the Auckland population census of 1981 we computed age-specific incidence rates for each racial group. NPC patients not normally resident in New Zealand were excluded.

There were 66 cases of NPC:

Ethnic group	*Men*	*Women*
Chinese	17	5
European	19	5
Pacific Island Polynesian	14	3
Maori	3	0
Total	53	13

The age-specific incidence rates for men are shown in the figure. There is a gradient from Europeans through Pacific Island Polynesian to Chinese throughout the age range. Further analyses are reported elsewhere.[5]

Few of the Polynesians in this report had been born in New Zealand, the migrants generally retaining the social and dietary patterns of the Pacific Islands. Exposure to salted fish in the diet in childhood is considered important in the genesis of NPC in Chinese;[7] this could also hold true for the Polynesians since this is popular food in the islands.

An early study of NPC in New Zealand yielded, as expected, crude incidence rates much higher for Chinese than Europeans, but the number of Polynesians with NPC was far greater than expected, given the small Polynesian population in New Zealand at the time. In retrospect, it appears that evidence for a high risk of NPC in Polynesians has been around for some time.

The migrant Polynesian in New Zealand possibly provides us with a unique opportunity to study racial and environmental factors in the genesis of this intriguing disease.

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CROSS-REACTIVE PROTEIN IN DUCHENNE MUSCLE

SIR,—Increased knowledge of the genetic and biochemical basis of Duchenne/Becker muscular dystrophy has led to improvements in diagnosis through DNA and protein-based analyses. Indeed, a prognosis can now be accurately given in the absence of a family history by assays of dystrophin on small amounts of muscle coupled with an astute clinical assessment.[1-6] So far, published studies have used antisera to the mid-portion (rod domain) of the large dystrophin protein, and all have indicated that dystrophin deficiency (less than 3%) is specific for, and diagnostic of, severe Duchenne dystrophy. We have raised five additional polyclonal antisera against non-overlapping regions of human dystrophin (Koenig M, unpublished). These antisera, together with our well-characterised mouse 30 kD and 60 kD antisera,[1-4,6] recognise antigenic sites representative of most of the entire 400 kD dystrophin protein.

To extend our analysis of dystrophin abnormalities in patients with neuromuscular disease, six of the seven antisera (1–2, 30 kD, 60 kD, 9, 10, and 11) were affinity purified with the respective antigen covalently coupled to 'Affigel 10' (Biorad). Anti-trpE antibodies were then immunoabsorbed from each affinity-purified antibody preparation, such that each preparation was specific for a distinct region of the dystrophin protein. These preparations were then tested on muscle with both immunofluorescent and immunoblot techniques.

When used for immunofluorescent detection of dystrophin in cryostat sections of muscle, all antibody preparations replicated the previously published results with the 30 kD and 60 kD antibodies: the characteristic peripheral immunostaining of dystrophin was deficient in Duchenne muscular dystrophy (DMD) (fig 1). However, immunoblot studies gave equivocal results: a protein of 400 kD was detected in muscle from DMD patients by antibodies 1–2 and 10 but not by 30 kD, 60 kD, 9, and 11.

Two possible explanations for his paradoxical finding are that the 400 kD protein represents a hitherto uncharacterised product of the DMD gene or that it is an unrelated protein of the same apparent molecular weight as dystrophin recognised specifically by antibodies 1–2 and 10. To distinguish between these two possibilities, we identified a DMD patient who had a deletion which resulted in the absence of all detectable exons of the dystrophin gene (fig 2). The muscle of this patient was tested by immunoblot analysis with the six antibody preparations. As with other Duchenne muscle specimens, a protein which co-migrated with dystrophin was detected with antibodies 1–2 and 10, but not with 60 kD, 30 kD, 9, or 11 (fig 3). Since this patient lacks the entire dystrophin gene, yet can still produce the 400 kD protein, we conclude that this protein is not a product of the dystrophin gene

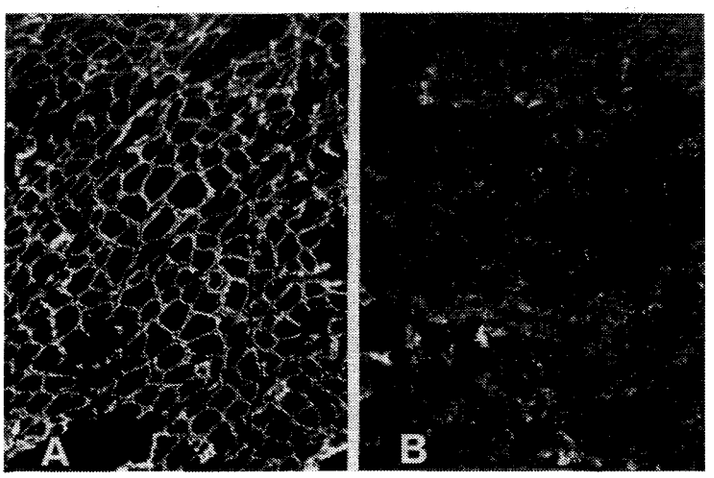

Fig 1—Immunofluorescence analysis of muscle from a limb-girdle patient (A) and patient with DMD (B).

Peripheral dystrophin immunostaining is detected in (A) but not (B). The antibody preparation used was affinity-purified "anti-10", raised against carboxy terminus of dystrophin (DMD muscle in [B] is from same patient illustrated in figs 2 and 3.)

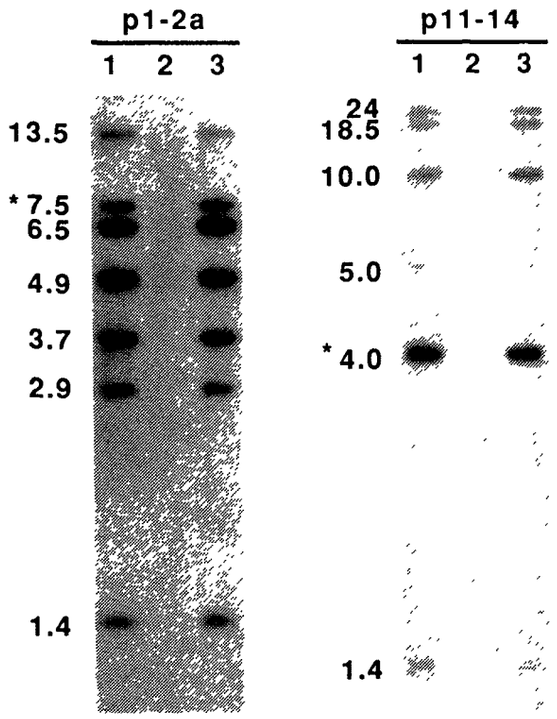

Fig 2—Identification of deletion for entire dystrophin gene.

DNA (lane 2) showing absence of hybridisation with dystrophin gene probe (normal pattern lanes 1 and 3). Autoradiographs are of *BglII* digested DNA probed with dystrophin cDNA corresponding to 5′ (p1–2a) and 3′ (p11–14) end of dystrophin gene. Sizes (kilobases) of exon-containing fragments are indicated, and the first and last exons are labelled with an asterisk.[7] Ethidium-bromide-stained gels (not shown) confirmed equal loading of DNA in all lanes. PCR analysis with primers for several exons, including numbers 1 and 60, also failed to detect any dystrophin gene sequences in this patient (not shown).

but a cross-reactive protein detected by antibodies directed against the amino and carboxy terminal domains of dystrophin. This cross-reactive protein species was detected in *mdx* mouse muscle by the same two antibody preparations, and also in Becker muscular dystrophy muscle, where it was accompanied by the expected smaller molecular weight dystrophin. Analysis of tissues from *mdx* mice indicate that this cross-reactive protein is not muscle-specific (not shown).

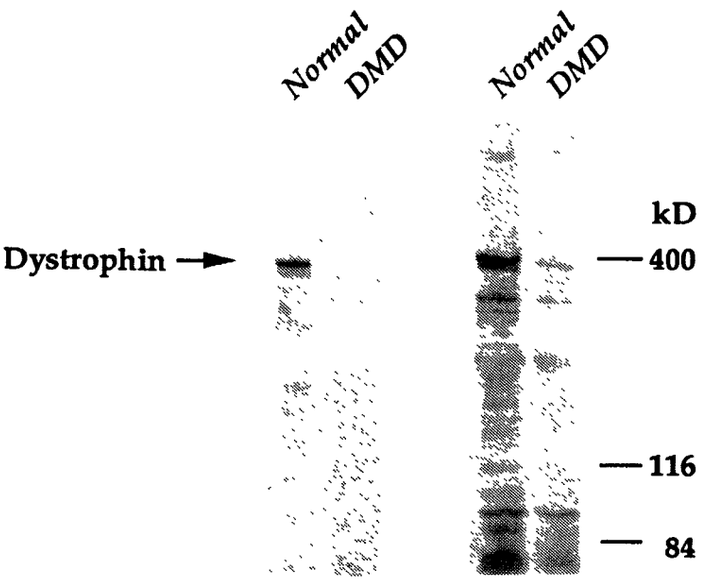

Fig 3—Identification of 400 kD protein species which co-migrates with dystrophin, yet is not product of dystrophin gene.

Immunoblot analysis of limb-girdle patient (lanes 1) and same DMD patient shown in fig 2 (lane 2) reveals a cross-reactive protein of molecular weight similar to that of dystrophin with antibodies 1–2 (right panel, lane 2) and 10 (not shown). This cross-reactive protein is not recognised by 30 kD antibodies (left panel, lane 2) or 60 kD, 9, or 11 antibodies (not shown).

Antisera raised against the amino and carboxy termini may not therefore be useful for immunoblot analysis of muscle because a cross-reactive protein which co-migrates with dystrophin will complicate interpretation. This cross-reactive protein does not seem to co-localise with dystrophin at the plasma membrane (fig 1), making it possible to use all antibody preparations for immunofluorescence analysis.

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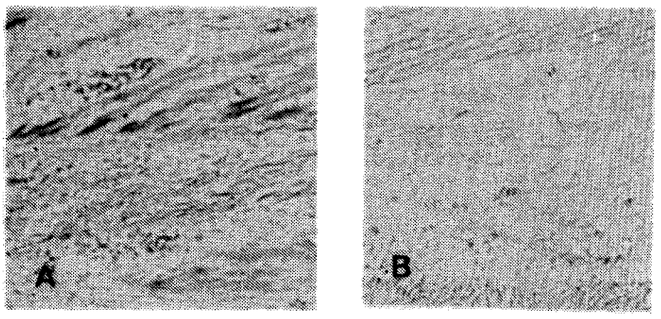
IMMUNOLOGICAL STUDY OF DYSTROPHIN IN DUCHENNE FETUS

SIR,—We have studied immunohistochemically a 12-week-old fetus, aborted after haplotyping had revealed a high risk of Duchenne muscular dystrophy (DMD). Southern blotting and field inversion gel electrophoresis[1] revealed a duplication in the middle of the DMD gene, that had caused severe Duchenne dystrophy in three relatives. This indicates a frameshift in the gene, producing truncated and unstable dystrophin.[2] Because the question of the presence of dystrophin in at-risk fetuses below 20 weeks of age has never been addressed, we studied sections of leg tissue immunohistochemically.

We used polyclonal antibodies directed against the 60 kD (1·3–2·7 kb of cDNA) and 30 kD (3·7–4·4 kb) segments of the NH_2-proximal half of dystrophin[3] and the COOH-terminal 17 aminoacids (11·212-11·263 kb) of dystrophin. The 60 kD and 30 kD antibodies clearly stained the ends of the myotubes in skeletal muscle of both a normal fetus (not shown) and the Duchenne fetus (figure, A) between the distal nuclei and myotendinous junction,



Dystrophin expression in 12-week DMD fetus.

Tissue was fixed and stained[5] and sections were immunostained with 30 kD antibody (A) or COOH terminus antibody P1461 (B).