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PTEN mutations are uncommon in Proteus syndrome

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EDITOR—Proteus syndrome (MIM 176920) is a rare, congenital, hamartomatous disorder, which is a member of a group of local overgrowth diseases. Happle[1] proposed that some of these disorders are the result of the action of a lethal gene that can only survive in the mosaic state, which arises from an early somatic mutation or from a half chromatid mutation. Such a mechanism has been shown to be the underlying basis of McCune-Albright syndrome (MIM 174800).[2] One of the mandatory diagnostic criteria for Proteus syndrome is a mosaic distribution of lesions and sporadic occurrence, entirely consistent with Happle's hypothesis.

Currently, little is known about the molecular causes of Proteus syndrome. It is, however, likely that the overgrowth of tissue involves all germ layers. This may be because of hyperproliferation, an absence of appropriate apoptosis, or alternatively cellular hypertrophy. There have been few investigations into the molecular basis of Proteus syndrome. Zhou *et al*[3] recently identified *PTEN* mutations in a patient with a Proteus-like syndrome. Germline *PTEN* mutations are found in a high proportion of patients with Cowden (MIM 158350) and Bannayan-Riley-Ruvalcaba (BRR) syndromes (MIM 153480),[4–7] which share many features of Proteus syndrome. These observations make *PTEN* a strong candidate for a gene mutated in Proteus syndrome. To investigate this possibility, we examined eight patients with Proteus syndrome for *PTEN* mutations. All were unrelated and had classical Proteus syndrome using published diagnostic criteria.[8] Samples were obtained with informed consent and local ethical review board approval. Fibroblasts were cultured from skin biopsies obtained from normal tissue and from regions of overgrowth. Genomic DNA was extracted from cultured cells using a standard sucrose lysis technique. *PTEN* mutational analysis was performed by PCR based conformational specific gel electrophoresis using published oligonucleotides[9] and semi-automated sequencing using an ABI 377 Prism sequencer. A common exon 4 polymorphism was observed in three of the patients, but no missense or truncating mutations in any of the eight samples were detected, suggesting that mutation in *PTEN* is unlikely to be a common cause of Proteus syndrome.

We evaluated *PTEN* as a candidate gene because of its role in the overgrowth syndrome Cowden disease and the recent report of a *PTEN* mutation in a boy with Proteus-like syndrome.[3] PTEN plays a role in the regulation of PI3 kinase signalling, which is involved in the control of apoptosis and cell cycle progression.[10] Hence, by removing the regulatory effects of PTEN on PI3 kinase signalling, deregulated cellular growth could occur. PTEN also appears to play a role in the regulation of cell size and a role for the PI3 kinase signalling pathway in the determination of organ size in mammals has been reported.[11] The boy reported by Zhou *et al*[3] with Proteus-like syndrome had a germline single base transversion resulting in an Arg 335 to Ter substitution in *PTEN*. A second *PTEN* mutation resulting in Arg 130 to Ter was found in DNA from a naevus, lipoma, and an arteriovenous mass. The authors postulated that the first germline mutation gave rise to many of the features of BRR and that the second hit occurred early in embryogenesis causing mosaicism. In our study we did not detect *PTEN* mutations in any of the Proteus syndrome patients we examined. Zhou *et al*[3] similarly failed to detect any *PTEN* mutations in five patients with classical Proteus syndrome; their patient with *PTEN* mutations did not fulfil the stringent diagnostic criteria for Proteus syndrome.

Mutations in the coding region of *PTEN* do not appear to be implicated in classical Proteus syndrome. *PTEN* may still be involved, as our finding does not preclude the possibility that it may be aberrantly imprinted in Proteus syndrome, for example by promoter methylation,[12] leading to reduced PTEN expression. Given the innumerable possibilities for a molecular basis of Proteus syndrome, the identification of which genes are disrupted will prove difficult. One strategy for dissecting the molecular pathways of Proteus and other overgrowth syndromes is through examining the expression patterns of genes in affected and unaffected tissues, which is becoming feasible with the advent of microarray technology.[13]

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Limited contribution of interchromosomal gene conversion to *NF1* gene mutation

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EDITOR—Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorders with a population frequency of 1 in 3500.[1] The disease is clinically characterised by multiple neurofibromas, café au lait spots and Lisch nodules of the iris. The *NF1* gene, a tumour suppressor gene, resides on the proximal long arm of chromosome 17 (17q11.2). It spans approximately 350 kb of genomic DNA and, comprising 60 exons, encodes the protein neurofibromin.[2] This protein, consisting of 2818 amino acids, contains a central domain that has homology with GTPase activating proteins (GAPs).[3]

A distinct feature of the *NF1* gene is the very high spontaneous mutation rate (1×10^{-4} per gamete per generation), which is about 100-fold higher than the usual mutation rate for a single locus.[1] Up to 50% of all NF1 cases are thought to result from de novo mutations. The *NF1* gene provides a large target for mutations because of its relatively large size, but this may only account for a factor of 10 in terms of increase in mutation rate.[4] The presence of numerous *NF1* pseudogenes has been proposed as an explanation for the high mutation rate in NF1.[5] In the human genome, at least 12 different *NF1* related sequences have been identified on chromosomes 2, 12, 14, 15, 18, 21, and 22.[5–13] Most of the *NF1* pseudogenes have been mapped in pericentromeric regions. The chromosome 2 *NF1* pseudogene has been localised to region 2q21, which is known to contain the remnant of an ancestral centromere.[14] Owing to the absence of selective pressure, mutations may accumulate in the *NF1* pseudogenes. Consequently, the pseudogenes could act as reservoirs of mutations, which might be crossed into the functional *NF1* gene by interchromosomal gene conversion.[5] Gene conversion, the non-reciprocal transfer of genetic information between two related sequences, has been recognised as a mutational mechanism for several human genes.[15–17] In all these cases, the conversions occurred between gene and pseudogene on the same chromosome. For NF1, interchromosomal gene conversion is required as none of the *NF1* pseudogenes is located on chromosome 17. Interchromosomal gene conversion has been reported to occur between the von Willebrand factor gene on chromosome 12 and the von Willebrand pseudogene on chromosome 22.[18]

Gene conversion requires close contact between the functional gene and the corresponding pseudogene. The pericentromeric location of the functional *NF1* gene and its pseudogenes may enable this close contact since centromeres tend to associate with each other in a non-random fashion.[19 20] This is underlined by our finding that the *NF1* pseudogenes on chromosomes 2, 14, and 22 have arisen by repeated transposition events between (peri)centromeric locations on these chromosomes (Luijten *et al*, submitted).[13] However, the high mutation rate in NF1 cannot be explained exclusively by interchromosomal gene conversion. Only a small part of the functional *NF1* gene is represented in the *NF1* pseudogenes (see below), while *NF1* gene mutations are scattered over the entire gene. In this study, we investigated whether interchromosomal gene conversion contributes to the mutation rate in NF1.