hypopigmentation in PWS also correlates with *P* deletion.[7] A hemizygous deletion of *P* cannot completely explain the mild degree of hypopigmentation more commonly seen in AS or PWS because heterozygous carriers of severe autosomal recessive OCA2 patients do not usually have hypopigmentation. Therefore, one can speculate that a pigmentation modifier gene(s) may be present in the deleted region of 15q11-q13.

The A481T mutation has previously been found in an African-American patient with *P* related autosomal recessive ocular albinism,[3] as well as in one of 50 unrelated controls.[4] The frequency of OCA2 is approximately 1/40 000 and 1/10 000 in whites and in African-Americans, respectively.[1 12] Carrier frequency is thus estimated to be 1/50-1/100. The carrier frequency of A481T reported by Lee *et al*[4] may have been overestimated, since previous studies have not shown that A481T is a predominant mutation in OCA2.[3 12 13]

The function of the A481T allele was shown by transfecting A481T mutant *P* cDNA into *p* null mouse melanocytes,[14] which showed that the A481T allele had approximately 70% function of the wild type allele. Therefore, since our patient is hemizygous, he may only have 35% of *P* gene function in melanin production compared with the wild type.

Our patient showed a severe deficiency of pigmentation during infancy to early childhood, but melanin production gradually increased and he had dark hair by 12 years of age. This is in keeping with the natural history of OCA2, as gradual increase in pigmentation during childhood is commonly seen in OCA2 patients. These findings are also consistent with the finding that the A481T allele has the potential for producing significant amounts of melanin as shown in the tissue culture experiments. Many mammalian genes are known to have an effect on pigmentation[15] and are developmentally controlled, although the precise mechanisms have not been established. Possible deletion of other pigmentation modifier gene(s) located in 15q may also explain the profound hypopigmentation in our patient during early childhood.

In conclusion, we describe the first genetic evidence that the *P* gene mutation is responsible for OCA2 associated with AS. These findings increase the spectrum of clinical conditions (AS or PWS plus OCA2, OCA2, ocular albinism) associated with *P* gene mutations.

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Anticipation in progressive diaphyseal dysplasia

EDITOR—Progressive diaphyseal dysplasia (MIM 131300) is an autosomal dominant osteochondrodysplasia with late onset and marked variability in expression of clinical and radiological features.[1] We have recently described a patient with this diagnosis and suggested the presence of anticipation,[2] the tendency of a familial disorder to occur earlier in the younger than in the older generations of a family.[3]

In order to assess whether this hypothesis were true, we examined 24 other people from the same family (fig 1) who were invited and agreed to participate. We excluded the proband and tried to avoid ascertainment bias by including in the study all family members who were alive in four generations. Although the diagnosis of progressive diaphyseal dysplasia had not been previously recorded in any of them, some family members had assigned to themselves the status of affected or unaffected. This was often discordant with the clinical and radiological data. It is also likely that people with bone complaints were more eager to consent to medical observation.

All family members were personally examined. The presence or absence and the age of onset of pain in the limbs, easy fatiguability, headache, poor appetite, and difficulty in running were recorded. Height, weight, and OFC were determined. Skull, spine, and limb morphology was examined and exophthalmos, muscle mass and strength, joint movement, tendon reflexes, and walking were assessed. Bone radiographic surveys, haemoglobin, white blood cell count, erythrocyte sedimentation rate, and serum alkaline phosphatase were performed.

The diagnosis of progressive diaphyseal dysplasia was clinically and radiologically established in 15 family members of the proband (table 1). Five of these have been previously described.[2]

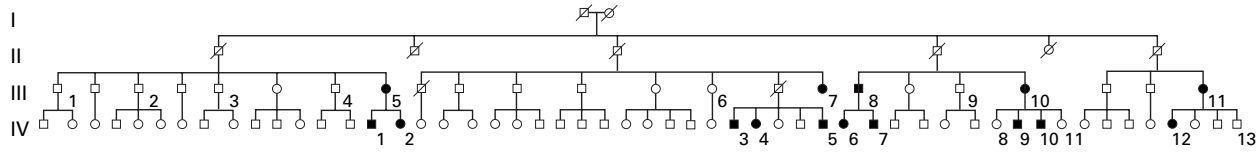

Figure 1 Pedigree showing 15 cases of progressive diaphyseal dysplasia in generations III and IV of one family. Only those personally examined are numbered and included in the statistical analysis.

Table 1 Clinical, laboratory, and radiographic data from 15 patients with progressive diaphyseal dysplasia

	Patient														
	III.5	*III.7*	*III.8*	*III.10*	*III.11*	*IV.1*	*IV.2*	*IV.3*	*IV.4*	*IV.5*	*IV.6*	*IV.7*	*IV.9*	*IV.10*	*IV.12*
Age at onset of symptoms (y)	30	20	20	15	21	15	14	3	10	10	8	5	18	13	2
Age at examination (y)	54	64	51	42	47	25	16	36	34	29	23	8	20	16	26
Pain in limbs	PMo	PMi	PMi	PMi	PMi	PMi	PMi	PMo	PMo	PMo	PMo	PMi	A	A	PS
Easy fatiguability	PMo	PMi	A	PMi	A	PMi	A	PMo	PMi	PMo	PMo	A	A	PMi	PMo
Headache	A	A	A	PMi	A	A	PMi	A	PMi	A	A	A	PMi	A	A
Poor appetite	A	A	A	PMi	A	PMi	A	A	A	A	A	A	A	A	PMi
Poor muscle mass	A	A	A	A	A	A	A	PMi	A	PMo	A	PMi	A	A	PMo
Waddling gait	PMi	PMi	A	A	A	PMi	A	PMi	PMi	PMo	PMo	A	A	A	PS
Radiographic bone changes	PMo	PS	PMo	PMo	PMi	PMo	PMi	PS	PS	PS	PS	PMi	PMi	PMi	PS
Haemoglobin (g/dl)	13.6	13.2	14.9	12.9	12.9	15.3	13.3	13.7	14.5	14.6	13.3	12.8	13.9	14.7	13.7
Erythrocyte sedimentation rate mm/h	14	28	1	3	11	9	11	13	16	15	6	11	10	4	16
Alkaline phosphatase (U/l)	27	42	81	24	33	28	20	35	52	57	203	105	46	71	97

P, present; A, absent; S, severe; Mo, moderate; Mi, mild.

We were able to determine the age of onset of the disease in these 15 patients (table 1), five from generation III and 10 from generation IV. Age of onset of the disease was defined as that of the initial symptom. The earlier age of onset in the younger generation was statistically significant (table 2). A similar analysis applying the same criteria to the data previously described in another extended family with progressive diaphyseal dysplasia[4] gave identical results (table 2). Other published pedigrees do not describe large families and often do not mention the age of onset of the disease.[5–10]. However, the available data, excluding once again the probands, is in accordance with the previous results (table 2).

A previous suggestion that anticipation was enhanced in father to son transmission[2] is not supported by these new data; the patient with the earliest age of onset and one of the most severely affected is a female (IV.12, fig 1, table 1), who inherited the disease from her mother.

As has been previously pointed out, statistical anticipation without biological anticipation would occur if the disorder reduces fertility.[3] However, the mean number of children in the family members of generation III is 2 for those that are healthy and 2.8 for those that are affected. The small number of people of generation IV who have already had children does not merit consideration.

Another type of bias could be that some of the younger persons might be classed as unaffected but might develop the disease later. This would inevitably increase the mean age of onset as assessed in the younger generation. However, the reported ages of onset of progressive diaphyseal dysplasia are between 4 and 32 years and in 25 patients all but four had ages of onset younger than 20, the exceptions being the ages of 21, 25, 30, and 32 years old.[2 4] In order to avoid this bias, we did not include the data on three persons, one of whom is affected, from generation V. The ages of the unaffected persons from generation IV are 16, 23, and 24 years.

Table 2 Mean age of onset of progressive diaphyseal dysplasia in the older and younger generations in the family described here (family 1), the family reported by Naveh et al[4] (family 2), and other pedigrees[5–10]

		Number	*Mean*	*SD*	*p*
Family 1	Older generation	5	21.2	5.4	
	Younger generation	10	9.8	5.3	0.001903
Family 2	Older generation	5	18.0	10.2	
	Younger generation	5	4.2	3.1	0.020079
Families 1 and 2	Older generation	10	19.6	7.9	
	Younger generation	15	7.9	5.3	0.000195
Other pedigrees	Older generation	7	21.4	10.5	
	Younger generation	5	3.0	1.9	0.003419

Although anticipation was only accepted as a true biological concept in the last decade, the list of conditions exhibiting it is growing rapidly.[3 11] Most of them are neuropsychiatric diseases and trinucleotide repeat expansions are usually suggested to be the cause.

This report suggests that anticipation occurs in progressive diaphyseal dysplasia and widens the disease spectrum of this concept to bone dysplasias. A dynamic mutation with trinucleotide repeat expansion may or may not be the cause of this osteochondrodysplasia of dense bone with unknown pathogenesis. The search for the molecular explanation of this and other rare genetic disorders that cause dense bones could disclose approaches to more common health problems such as osteoporosis.[12]

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Hereditary neuropathy with liability to pressure palsies: two cases with a reciprocal translocation t(16;17)(q12;p11.2) interrupting the *PMP22* gene

EDITOR—Hereditary neuropathy with liability to pressure palsies (HNPP) or tomaculous neuropathy is an autosomal dominant disease.[1] HNPP patients present with acute or recurrent transient muscle palsies and paraesthesias, usually after minor trauma. HNPP is characterised from the pathological point of view by the presence of sausage shaped swelling of the myelin sheath or tomacula[2] in sensory and motor nerves.[3]

A deletion on the proximal short arm of chromosome 17 was detected in affected members of HNPP families.[4 5] The deleted region is the same as that duplicated in Charcot-Marie-Tooth disease type 1 (CMT1A). While the CMT1A phenotype is mostly the result of extra dosage of the genes contained in this duplicated region, the HNPP phenotype results from monosomy of the same region in over 85% of cases.[6] This duplication/deletion event is thought to occur because of the unequal crossing over between two large repeats bordering the CMT1A region,[7] which encompasses 1.5 Mb of 17p11.2.[8]

The association between CMT1A and the peripheral myelin protein 22 (*PMP22*) gene, located within the CMT1A duplicated region, was established by the finding of point mutations in this gene[9 10] in CMT1A patients. The *PMP22* gene spans approximately 40 kb and contains four coding and two 5' untranslated (1A and 1B) exons.[11] In 35 unrelated patients with inherited peripheral neuropathies, 27 mutations in the *PMP22* gene have been described, affecting the different domains of the protein,[12] some of them leading to a truncated protein. The clinical phenotypes of the patients carrying mutations in the *PMP22* gene are variable, giving some clues about correlations between phenotype and genotype. Five mutations in the *PMP22* gene have been described that result in a HNPP phenotype: two frameshift mutations,[13 14] two nonsense mutations,[15 16] and a splice site mutation.[17]

We report here a pedigree with two affected members (mother and son) with HNPP, both of whom carry a reciprocal translocation t(16;17)(q12;p11.2), which we have studied by FISH. The breakpoint on chromosome 17 in both patients lies within exons 1a to 3 of the *PMP22* gene.[11]

Table 1 Electroneurographic findings in two patients with hereditary neuropathy with liability to pressure palsies (HNPP)

	Motor and sensory nerve conduction					
	Ulnar nerve		*Median nerve*		*Peroneal nerve*	
	P	*M*	*P*	*M*	*P*	*M*
MCV (m/sec)						
R	40	40	53	60	—	27
L	39	48	52	44	32	31
N	(>52)		(>52)		(>48)	
DL (m/sec)						
R	3.5	2.8	4.5	4	—	—
L	3.2	3	3.7	3.8	—	—
SCV (m/sec)						
R	35	43	27	33	—	—
L	38	37	37	34	—	—
SAP amplitude (μV)						
R	2	4	2	2	—	—
L	3	2	3	3	—	—

P, proband; M, mother; R, right; L, left; N, normal value; MCV, motor conduction velocity; DL, distal latency; SCV, sensory conduction velocity; SAP, sensory action potential; —, not done.

The proband is a 24 year old male referred to our clinic for molecular analysis to confirm the clinical diagnosis of HNPP. The mother confirmed delayed milestones during infancy, followed by clumsiness and difficulties with running and schooling during childhood and adolescence. At the age of 21, he had two episodes of numbness and muscular weakness of the left arm following compression while sleeping and weight lifting, without full recovery. Clinical examination showed distal muscle atrophy and weakness of the left arm involving the long distal extensors of the upper limb, the abductor of the thumb, and the supinator of the arm. He was able to walk on his toes and heels and had normal reflexes. No other signs of muscle weakness were noted. Nerve conduction studies showed delayed and reduced sensory action potentials with prolonged motor latencies and slow motor conduction velocities (table 1). Clinical and electroneurographic studies were also carried out in the parents and his twin sister. The mother was clinically asymptomatic, although she reported episodes of paraesthesias with complete recovery involving the upper limbs. Electroneurographic findings were consistent with peripheral neuropathy (table 1) and the diagnosis of HNPP was established in the family. No other members were affected.

Chromosome spreads were obtained from peripheral blood of each patient and a G banding karyotype was performed. Conventional cytogenetics showed the presence of a reciprocal translocation between chromosomes 16 and 17 (t(16;17)(q12;p11.2)). FISH analysis was performed as described elsewhere[18] using YAC 181g9 (CEPH) and cosmids c49-E4 (GDB 437232) and c103-B11 (GDB 437233) (both cosmids kindly provided by Dr P I Patel) as probes. FISH analysis showed that YAC 181g9, which encompasses marker D17S122 to marker D17S879 and contains *PMP22*, crossed the translocation breakpoint. FISH with cosmids c49-E4 and c103-B11 showed that the breakpoint was within cosmid c49-E4, which contains the genomic region encoding the first three exons of the *PMP22* gene (fig 1). Therefore, we concluded that the *PMP22* gene[11] is interrupted in these two patients with the HNPP phenotype.

This is the first case of HNPP being caused by a reciprocal translocation that interrupts the *PMP22* gene. Cytogenetic studies and FISH confirmed the diagnosis in the patient. The two cases presented here not only confirm that the HNPP phenotype was the result of the interruption of the *PMP22* gene, but also show the variable penetrance of the phenotype in two related patients carrying the same mutation. It is therefore unlikely that other genes within the commonly deleted region have contributed to the phenotype. This suggests that modifiers in other genomic regions are involved in the clinical variability of peripheral neuropathies.

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