

to the classical definition of dominance which states that homozygotes and heterozygotes for a defect are phenotypically indistinguishable³⁻⁵. Instead, they display incomplete dominance, indicating that the normal allele may play a role in ameliorating the disease process. The *D4S10* locus, defined by the probe G8 and linked to the gene for Huntington's disease (HD), has permitted us to identify individuals with a high probability of being homozygous for this autosomal dominant neurodegenerative disorder⁶⁻⁹. These homozygotes do not differ in clinical expression or course from typical HD heterozygotes. HD appears to be the first human disease of genetically documented homozygosity that displays complete phenotypic dominance.

The HD gene was localized, in part, by study of the largest known HD family in the world, living along the shores of Lake Maracaibo, Venezuela¹⁰⁻¹³. The pedigree currently numbers almost 7,000 individuals: over 100 living symptomatic individuals and almost 900 family members born at 50% risk for carrying the gene defect but who are as yet asymptomatic. Among present generations of the pedigree, there are six unions between two symptomatic individuals, but there is nothing clinically unusual in their 31 offspring. We have concentrated our efforts on one large nuclear family, the 'H' family, in which both parents are living and many relatives could be assigned unequivocal genotypes.

To minimize the potential for bias in evaluating them, potentially homozygous offspring in the H family have been compared with 93 affected individuals, including 6 with juvenile onset of symptoms, and 307 'at-risk' relatives living in the same environment. Neurological, intellectual and behavioural function, as described in Fig. 1 have been assessed annually since 1981¹⁴.

Six years ago 1 of 14 children was already mildly symptomatic and in 1985 a second member of the sibship was diagnosed with HD after displaying 'soft signs' for the previous five years. The age of onset and progression of symptoms in these two is typical compared to those in other HD patients in Venezuela and the United States^{14,15}. In 1986, 7 siblings had soft signs and 5 remained neurologically normal.

The potential effects of homozygosity for the HD gene on intellectual function were also evaluated. A subset of the Venezuelan pedigree has been evaluated annually with a battery of neuropsychological tests assessing cognitive ability, memory and visual-motor dexterity¹⁶⁻²⁰. The offspring of the H family have not shown any unusually precipitous decline on neuropsychological tests. The H family is also typical with respect to the behavioural disturbances which are often noted in HD, as assessed through structured and unstructured interviews²¹⁻²³.

The *D4S10* locus was originally defined by the G8 probe, a 17-kilobase (kb) fragment of genomic DNA that detects two polymorphic *Hind*III sites when hybridized to human genomic DNA^{6,8}. A number of additional restriction fragment length polymorphisms (RFLPs) have been found using subclones of G8 or of R7, a second genomic clone overlapping G8^{7,9}. The probes used in this study, along with the polymorphic sites and haplotypes they define, are shown in Fig. 2. Results from typing the H family for these sites are shown in Fig. 1a and b.

The HD gene usually segregates with the C1 haplotype at the *D4S10* locus in the Venezuelan pedigree. Reconstruction of the *D4S10* haplotypes of all 4 grandparents of the H sibship confirms that each H parent inherited the HD gene on a chromosome with the C1 haplotype. In addition, the mother received the A3 haplotype and the father the A5 haplotype from their respective unaffected parents. In the absence of recombination, their offspring homozygous for the HD gene would also be homozygous for the C1 haplotype at the *D4S10* locus.

All four possible *D4S10* genotypes are represented among the 14 children of the H family. Four received two copies of the C1 haplotype, suggesting they are homozygous at the HD locus. Six children received one copy of the C1 haplotype, making them probable heterozygotes for the HD gene, and the remaining 4 children did not inherit the C1 haplotype. This

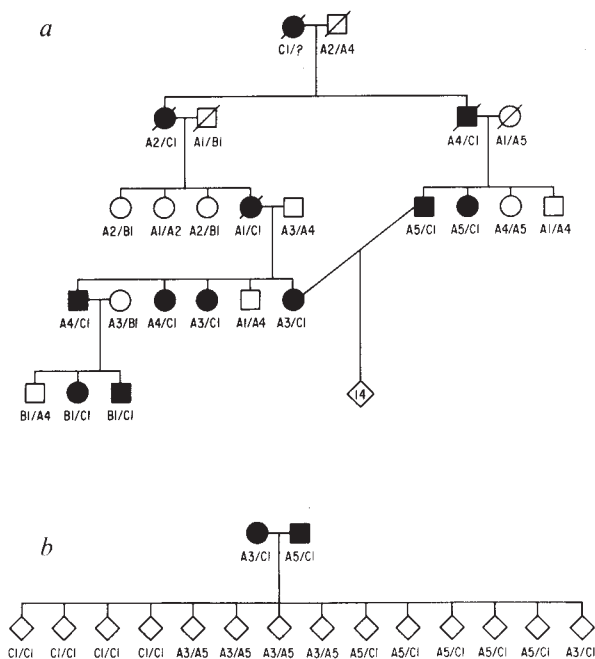


Fig. 1 Phenotypic assessment and segregation of *D4S10* and HD in the H family. *a*, Individuals from five generations of the H pedigree were typed for RFLPs at the *D4S10* locus. Family members diagnosed with HD are shown by darkened symbols and deceased individuals are denoted by a slash through the symbol. (Symbols are ○, female; □, male; ◇, one or more individuals, sexes not shown.) Haplotypes for *D4S10* were assigned as in Fig. 2. Genotypes were inferred from the phenotypic data after deducing the coupling phase of the various alleles for the *D4S10* RFLPs. In many cases, additional related individuals not shown here (for example, spouse and children) helped to confirm the assigned coupling phase. *b*, Inferred genotypes for the progeny of the HD by HD mating from *a*. The exact birth order of the children and the sex and HD status of each are not given to maintain confidentiality. Each C1/C1 individual has an approximately 92% probability of being homozygous for the genetic defect resulting in Huntington's disease.

Methods. The parents of the H family are second cousins once removed. The mother states that she is approximately 58 and her partner 63 years of age; no birth records exist. Each has been symptomatic for about 15 years. The mother began her pregnancies in her mid-teens with two miscarriages. In close succession, the couple had 15 children, including two sets of twins, one set of fraternal male twins and the other female. A female twin died at age 5 of gastroenteritis, leaving 14 living children available for study, ranging in age from 16 to 42 years. Blood samples from both parents, all 14 children and several other close relatives have been tested with more than 20 red-blood-cell and plasma markers and more than 50 polymorphic DNA markers to eliminate the possibility of non-paternity. Diagnosis of HD was based solely on the presence of characteristic extrapyramidal motor abnormalities. Behavioural disturbances were noted but were not themselves considered sufficient. The same neurologist (A.Y.) examined all members of the H family each year, joined in some years by between one and three other neurologists. Using the same rating scale, the neurologists demonstrated high inter-rater reliability in scoring³². The neurological examination assesses ocular motor ability, motor impersistence, fine motor coordination, tendon reflexes, and abnormal movements and postures such as chorea, dystonia and parkinsonian symptoms¹⁴. Functional capacity in patients was also rated³³. About one-third of the at-risk individuals examined displayed soft signs, minor neurological irregularities in one or more of the areas assessed. A diagnosis of HD was only made when unequivocal signs were present. Diagnostic feedback is not given unless requested, which almost never happens. Research participants correctly understand that the study is long-range and that its goals are to learn the cause of the illness and to devise therapeutic interventions.

distribution of progeny genotypes is consistent with mendelian segregation, assuming equal viability. It differs significantly ($P < 0.01$) from the distribution expected if HD homozygotes die prenatally.

We cannot be absolutely certain that the 4 children homozygous for the C1 haplotype are homozygous for the HD gene because of the possibility of recombination in the gametes of either or both parents. The genetic distance between the two loci is ~4 centimorgans (4% recombination, 1 lod confidence interval = 1–8%). Consequently, each child homozygous for the C1 haplotype has an ~92% chance of being homozygous for HD. The probability that none of the 4 is homozygous for the defect is $(0.08)^4$ or 4×10^{-5} suggesting that homozygosity at the HD locus does exist in this family and is not lethal *in utero*.

We have chosen to omit exact correlations between the genotypes in Fig. 1b and the personal and neurological data on individual members of the sibship, but this does not affect the conclusions to be drawn from this study. First and foremost, we wish to protect the confidentiality of all family members, particularly those found to be homozygous. Of the 14 offspring, 10 are destined to become ill but 4 should remain normal. The fact that children of HD homozygotes are inevitably HD-gene carriers makes privacy all the more essential. A second reason for avoiding identification of the individual genotypes in this family is our desire to continue the prospective study with investigators in the field remaining blind to the genetic status of each subject.

Among the 4 potential homozygotes for the HD gene there is at least one representative of each of the three diagnostic categories: definitive HD, displaying soft signs, and neurologically normal. There appear to be no phenotypic differences, either in age of onset, symptoms, or progression of illness, between an affected probable HD homozygote and the typical HD heterozygotes from the Venezuelan kindred or any US sample of patients. Similarly, a potential HD homozygote who displays soft signs or is neurologically normal cannot be distinguished clinically from other at-risk individuals who are potential HD heterozygotes. In terms of the phenotypes currently being monitored by neurological and psychological examinations, HD displays complete dominance. This permits two conclusions concerning the motoric, cognitive and psychiatric symptoms of this disorder: first, they are not influenced by dosage of the defective gene; and second, they are not mitigated by the normal allele.

Matings between two heterozygotes for a dominant disease are so rare that the opportunity to study large numbers of progeny from such unions is exceptional^{13,24–27}. In virtually every instance in which children are conceived between two heterozygotes, presumed homozygotes are quantitatively and sometimes qualitatively more severely affected than typical heterozygotes^{3,24–27}. The only possible exception to this rule described previously involved a family with Best's disease, or hereditary macular degeneration²⁸, where unfortunately, homozygosity could not be confirmed genetically.

In view of the obvious dose effects displayed by putative homozygotes in other dominant diseases, our finding that homozygosity for the defective allele in HD does not result in a more severely affected phenotype than heterozygosity is surprising. The importance of this observation lies in the clues it may provide to the nature of the disease process and its primary lesion. Complete dominance is known to exist in *Drosophila* and is thought to be associated with mutations causing a 'gain of function'^{29–31}. Clearly, HD cannot involve elimination of an essential enzyme activity as homozygosity is not lethal. The defect may compromise an enzyme activity that must exceed a strict threshold. Alternatively, the mutation might confer a novel property on a structural or regulatory protein, as the simple elimination of normal function would be expected to show a dosage effect or even lethality. If the HD defect causes a quantitative alteration, then overproduction of a normal protein, or

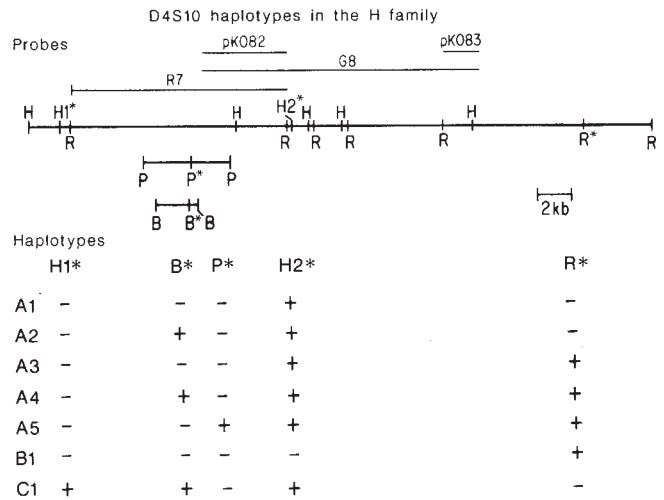


Fig. 2 *D4S10* haplotypes in the H family. A map of the polymorphic sites (denoted by *) and the probes used to detect them is shown (above) with definition of *D4S10* haplotypes (below); G8 is an insert of single-copy human DNA, from the terminal region of the chromosome 4 short arm, cloned in the phage vector Charon 4A^{6,7}. R7 is an overlapping genomic fragment also cloned in Charon 4A, and pK082 and pK083 are subclones of the indicated *EcoRI* fragments in pBR328. Two polymorphic *HindIII* (H) sites and one polymorphic *PstI* (P) site were detected using pK082 as a probe. Single polymorphic sites with *BglI* (B) and *EcoRI* (R) were monitored by probing with R7 and pK083 respectively.

Methods. For RFLP typing, genomic DNA from each individual was digested with the indicated enzyme (New England Biolabs), fractionated by agarose gel electrophoresis, transferred to a nylon membrane (Genatran, Plasco Inc.), and hybridized with ³²P-labelled probe DNA as described⁶, except that probes were prepared by oligonucleotide priming³⁴. Genomic DNA was obtained from permanent lymphoblastoid cell lines established for each member of the family as previously described^{35,36}. The presence or absence of each site on a given chromosome was used to define haplotypes at the *D4S10* locus. When all sites were considered, 7 haplotypes were evident in this portion of the Venezuelan HD pedigree and the disease segregated with the C1 haplotype.

the inappropriate production of a protein usually limited to a different tissue or to a different time of development, may be more likely than underproduction of a protein.

'Gene therapy' as a possible treatment for inherited disorders has engendered considerable interest recently. The normal allele at the HD locus does not apparently alter disease expression, so particularly imaginative approaches to gene therapy may be required for this condition: merely introducing a second normal allele is unlikely to have beneficial effects. Gene therapy aimed at blocking expression of the mutant allele (for example, by antisense mRNA) or at the introduction of unrelated gene(s) capable of correcting the biochemical defect may be a more rewarding approach.

The identification of specific genes or novel drugs capable of preventing the deleterious effects of HD is unlikely until the disease gene has been cloned and characterized. An intensive effort is underway to isolate the HD gene based on its map position despite limited knowledge of the nature of the defect. Cultured cells from HD homozygotes are very valuable in certain cloning strategies because their DNA contains no normal allele. Tissue samples from these individuals may ultimately enable identification of the gene product responsible for HD. Delineation of the molecular defect has obvious clinical importance and may also provide new insights into the biology of the nervous system. Of greater fundamental interest, perhaps, is that elucidating HD at the molecular level will reveal for the first time a genetic mechanism in humans capable of producing a completely dominant phenotype.

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Mesoderm induction in early *Xenopus* embryos by heparin-binding growth factors

J. M. W. Slack, B. G. Darlington, J. K. Heath* & S. F. Godsave

Imperial Cancer Research Fund, Developmental Biology Unit, Department of Zoology, University of Oxford, Oxford OX1 3PS, UK
* Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QY, UK

In early embryonic development the basic body plan arises because cells in different regions become programmed to follow different developmental pathways. We have proposed that in the early amphibian embryo this process of regional specification arises from the action of three different inducing factors, or morphogens (refs 1-5 and Fig. 1), but we have not until now had any idea of their chemical nature. In this paper we report (1) that pure basic fibroblast growth factor (bFGF), at very low concentrations and with high specificity, closely mimics the effect of the ventrovegetal (VV) signal and (2) that the transmission of the natural VV signal can be blocked by heparin, suggesting that it may be a heparin-binding factor such as bFGF.

In the main series of experiments, explants of ectoderm were cut from the animal pole region of stage 8 *Xenopus* blastulae (about 1,024-2,048 cell stage⁶) and exposed to bFGF. At stage 8 the explants include about 50-100 cells, but the cell number continues to increase by cleavage divisions without growth. Control explants round up over about two hours to form spheres with the original pigmented surface exterior and the original blastocoelic surface reduced to a small 'yolk plug'. Over the next two days, they become wrinkled and form epidermis around the outer surface. The internal cells may also all become epidermal but often some remain undifferentiated (Fig. 2b, d)^{7,8}. Mesodermal cells are formed rarely, even then probably only because of occasional dissection errors which lead to inclusion of endodermal cells from the blastocoel floor. Treated explants also round up during the first two hours. At about 6 hours, when

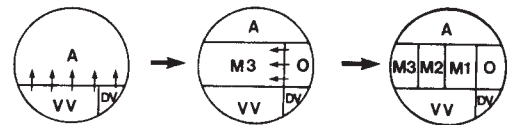


Fig. 1 Model of inductive interactions in the early amphibian embryo based on refs 1-5. The diagram depicts a blastula with the dorsal side to the right. Two mesoderm-inducing signals (DV and VV) are emitted from the vegetal hemisphere and induce an annulus of mesoderm consisting of a small organizer zone (O) and a large ventral mesoderm zone (M3). A dorsaling signal emitted by the organizer then induces intermediate structures such as somites and kidney (M1, M2) from the ventral mesoderm zone. Abbreviations: A, animal hemisphere ('ectoderm'); DV, dorsovegetal; VV, ventro-vegetal; O, organizer; M, mesoderm.

control embryos are middle gastrulae (stage 11-11½), they elongate with the original yolk plug at one end. The degree of elongation varies between batches of embryos and can range from a slight pear-shape departure from sphericity, to a sausage shape about twice as long as it is wide (Fig. 2a). The process is reminiscent of the normal gastrulation movements on the ventral side of the embryo. After about 24 hours, when control embryos are at tailbud stages, the treated explants begin to swell by fluid uptake and become vesicles several times their original volume (Fig. 2c). Specimens were fixed for histological or immunohistochemical examination at about 64 hours of culture (control stage 41).

The appearance depends on the concentration of bFGF used. Between about 2 and 30 ng ml⁻¹ the inductions closely resembled ventral-type mesoderms formed by explants from ventral or lateral marginal zones or by ventrovegetal-animal combinations^{1,2,4,5}. They consist of a concentric arrangement of loose mesenchyme, mesothelium and blood cells within an epidermal jacket and sometimes contain a few muscle cells (Fig. 2e, f). The blood cells do not usually differentiate sufficiently to synthesize haemoglobin but have a characteristic morphological appearance. Between 30 and 120 ng ml⁻¹, most of the explants contain some muscle and significant blocks of it are often produced (Fig. 2g). Their identity was confirmed by