Am. J. Hum. Genet. 59:1337–1342, 1996

A Translocation at 12q2 Refines the Interval Containing the Holt-Oram Syndrome 1 Gene

Jonathan A. Terrett,[1,2,*] Ruth Newbury-Ecob,[1,2] Nigel M. Smith,[2] Quan Yi Li,[1,2] Christine Garrett,[3] Phil Cox,[4] Damien Bonnet,[5] Stanislas Lyonnet,[5] Arnold Munnich,[5] Alan J. Buckler,[6] and J. David Brook[1,2]

[1]Department of Genetics, University of Nottingham, Queen's Medical Centre, and [2]Centre for Medical Genetics, City Hospital, Nottingham; [3]Kennedy Galton Centre, Northwick Park Hospital, Harrow; [4]Department of Pathology, Royal Postgraduate Medical School, Hammersmith Hospital, London; [5]Department de Pediatrie et Unite de Recherches sur les Handicaps Genetiques de l'enfant, Paris; and [6]Department of Neurogenetics, Massachusetts General Hospital, Charlestown

Summary

A gene for Holt-Oram syndrome (HOS) has been previously mapped to chromosome 12q2 and designated *HOS1*. We have identified a HOS patient with a de novo chromosomal rearrangement involving 12q. Detailed cytogenetic analysis of this case reveals three breaks on 12q, and two of these are within the HOS1 interval. By using a combination of chromosome painting and FISH with YACs and cosmids, it has been possible to map these breakpoints within the critical HOS1 interval and thus provide a focus for HOS gene–identification efforts.

Introduction

Holt-Oram syndrome (HOS) is a developmental disorder characterized by bilateral radial ray defects and a range of cardiac abnormalities including atrial septal defects (ASDs) and conduction defects (Holt and Oram 1960; Hurst et al. 1991; Newbury-Ecob et al. 1996). The gene causing HOS maps to chromosome 12q24 in a region of ~6 cM between sequence-tagged-site (STS) markers D12S84 and D12S79 (Bonnet et al. 1994). This interval is roughly equivalent to 6 Mb, and, in the absence of further recombination events in families, gene identification across such a large region is a major undertaking. Chromosomal rearrangements have proved vital to other positional cloning projects, including the discovery of the genes for Duchenne muscular dystrophy (Ray et al. 1985), neurofibromatosis type 1 (NF1; Viskochil et al. 1990), and campomelic dysplasia (Foster et al. 1994). Such rearrangements, associated with particular inherited disorders, provide a focus for gene-identification efforts. The cell line established from the HOS patient described here, (patient A) with a translocation on 12q, is thus a useful resource that will facilitate the identification of the HOS1 gene and more generally assist the ordering of markers in the region.

We have characterized the 12q translocation breakpoints in relation to the HOS1 interval, using cosmid and YAC clones in FISH experiments. During the course of this work, some of the YACs that are positive for STSs from the HOS interval have been found to contain material derived predominantly from chromosomes other than 12. The data from the cosmids and the YACs that contain mostly (or only) chromosome 12 material show that there are two breaks within the HOS1 interval. The location of the HOS gene can be refined to one of two intervals, either between CEPH mega-YACs 815f2 and 919a2 or within CEPH mega-YAC 887b9, distal to marker b023yd5.

Material and Methods

Ethical approval for this study was obtained from the Review Board, City Hospital NHS Trust, Nottingham, and consent for this study was given by the parents of patient A.

STSs

All PCR amplifications were carried out in 25-μl reaction volumes by using 50 ng of YAC DNA or 10 ng of cosmid DNA, 15 pmol of each primer, 0.25 mM of each dNTP, 50 mM KCl, 10 mM Tris.Cl pH 8.4, 1.5 mM $MgCl_2$, 0.01% gelatin, and 1 U *Taq* polymerase (Boehringer Mannheim). Reactions were overlayed with mineral oil and subjected to 30 cycles of 94°C for 40 s and 50°C for 30 s. PCR products were checked on 2% agarose gels containing 0.5 $\mu g/ml^{-1}$ ethidium bromide.

DNA Preparation and Labeling

Cosmid DNA was isolated by standard alkaline lysis (Birnboim and Doly 1979). YAC DNA was isolated

Received February 20, 1996; accepted for publication September 10, 1996.

Address for correspondence and reprints: Dr. J. David Brook, Department of Genetics, Queen's Medical Centre, University of Nottingham, Nottingham, NG7 2UH, United Kingdom. E-mail: david.brook@nottingham.ac.uk

*Present affiliation: Smithkline Beecham Pharmaceuticals, Essex.

0002-9297/96/5906-0021$02.00

from 5 ml Synthetic complete (Sherman 1991) overnight (30°C) cultures by using the glassbead miniprep method (Hoffman and Winston 1987). Inter-Alu PCR was performed on 50 ng YAC DNA as described by Nelson et al. (1989) by using primers Alu 1 5′ggattacagg c/t a/g tgagcca 3′ and Alu 2 5′a/g cc a c/t tgcactccagcctg 3′. Combined Alu 1, Alu 2, and Alu 1+2 PCR products (2 μg) or cosmid DNA (3 μg) were purified using Geneclean II (BIO 101). One to two micrograms of each DNA were labeled using the BioNick kit (Gibco-BRL), and labeled DNAs were purified with Geneclean II.

FISH

Metaphase spreads were made as by Verma and Babu (1989). Labeled probes were hybridized to metaphase chromosomes and detected by Avidin-FITC with one round of amplification as in Driesen et al. (1991). Visualization of chromosomes and detection of positive signals was performed on a Olympus BX50 camera and images "captured" using the Optivision Sirius VI system. Chromosome 12 material was "painted" with a commercially available kit (Cambio) according to the manufacturer's instructions.

Results

Clinical Evaluation

Patient A was the first pregnancy of healthy unrelated parents. The pregnancy was uneventful until a scan at 21 wk detected bilateral upper-limb abnormality. Termination of pregnancy was induced by cervagem following counseling at 21 wk, and a female fetus was delivered of size in keeping with the gestation. Postmortem examination showed shortening of both forearms, with the left more reduced than the right. The right hand showed acute radial deviation and syndactyly of the first and second digits. The left hand showed acute radial angulation and absence of the thumb. The shoulders were narrow. The lower limbs were normal, apart from minimal nonfixed talipes equinovarus deformity of the left foot. The heart showed the presence of a secundum ASD. X-rays confirmed clinical findings, showing a hypoplastic right radius and curved ulna, absent first metacarpal and phalanges. The left radius was absent, and the ulna hypoplastic. The shoulders were narrow with clavicular angulation. These findings are typical of HOS, in particular the asymmetry with greater severity of the left side, involvement of the shoulder girdle, and associated ASD (Newbury-Ecob et al. 1996). No other abnormalities were observed in the fetus. Neither parent shows any cytogenetic abnormality nor any HOS features. Radiographs of their hands and arms were examined, and electrocardiograms were performed.

FISH Analysis I: Normal Chromosomes

YACs were identified from the Généthon database or from Krautner et al. (1995) and subsequently tested for STS content before being used for FISH studies. YACs were labeled with biotin, hybridized to metaphase spreads from lymphocyte cultures of an unaffected individual, and detected fluorescently. Several of the YACs contain material derived predominantly from chromosomes other than 12q. These are YACs 910g1 (chromosome 3), 920g2 (chromosome 13), 955d8 (chromosome 1), 819f2 (chromosome 19), 722h7 (chromosome 12p), and 734h1 (chromosome 11). The YACs that gave good FISH signals on 12q—804b7, 887b9, 919a2, 815f2, and 747e2—were used to locate the translocation breakpoints. In addition, cosmid 747-A5 and cosmid 14 were mapped using FISH on both normal and translocation chromosomes.

The STS content of the YACs and cosmids is as follows: cosmid 747-A5 is positive for D12S84; YAC 747e2 is positive for D12S84 and D12S105; YAC 815f2 is positive for D12S105, D12S234, and 240we1; YAC 919a2 is positive for 291xe9, WI-8957, and WI-494 (D12S861); cosmid 14 is positive for b023yd5; YAC887b9 is positive for b023yd5, D12S129, WI-5732, D12S136, WI-9794, 312yb1, and D12S354; and YAC 804b7 is positive for 312yb1, D12S354, and D12S79.

FISH Analysis II: Translocation Chromosomes

A fibroblast cell line was established from a skin sample of patient A. The karyotype was determined by G-banding and refined using FISH analysis. FISH studies on metaphase chromosomes from this cell line produced fluorescing signals on four different chromosomes (see fig. 1*A*). This includes the normal chromsome 12, which is fully labeled, the der (12) chromosome containing material from 10q, the der (8) chromosome containing interstitial 12q material, and the der (2) chromosome, which appears metacentric with material from 12q on each arm (this der (2) chromosome is only slightly larger than the normal chromosome 12). On the basis of the G-band analysis and the painting data, the karyotype of patient A is defined as 46,XX, t (2;8;12) (2qter2q2-2.2::8p23.1→8p11.1::12q15→12q23::8p11.1→8qter; 8pter→8p23.1::12q23→12q24.31::2p15→2q22.2::12q2-4.31→12qte r), t (2;10) (2pter→2p15::10q11.2→10pter), t (10;12) (10qter→10q11.2::12q15→12pter).

The translocation products are represented diagramatically in figure 2. This indicates that three breaks (*x*, *y*, and *z*) are present on the q arm of one of the chromosome 12 homologues, producing three disjoined fragments (I, II, and III). Fine mapping of the translocation breakpoints has been achieved by using cosmid and YAC clones, which, from FISH studies on normal chromosomes, have been shown to map to 12q and which are positive for STS markers mapping to the HOS1 region. Figure 1*B* shows that the most proximal region of 12q (fragment I), which forms part of the der (8)

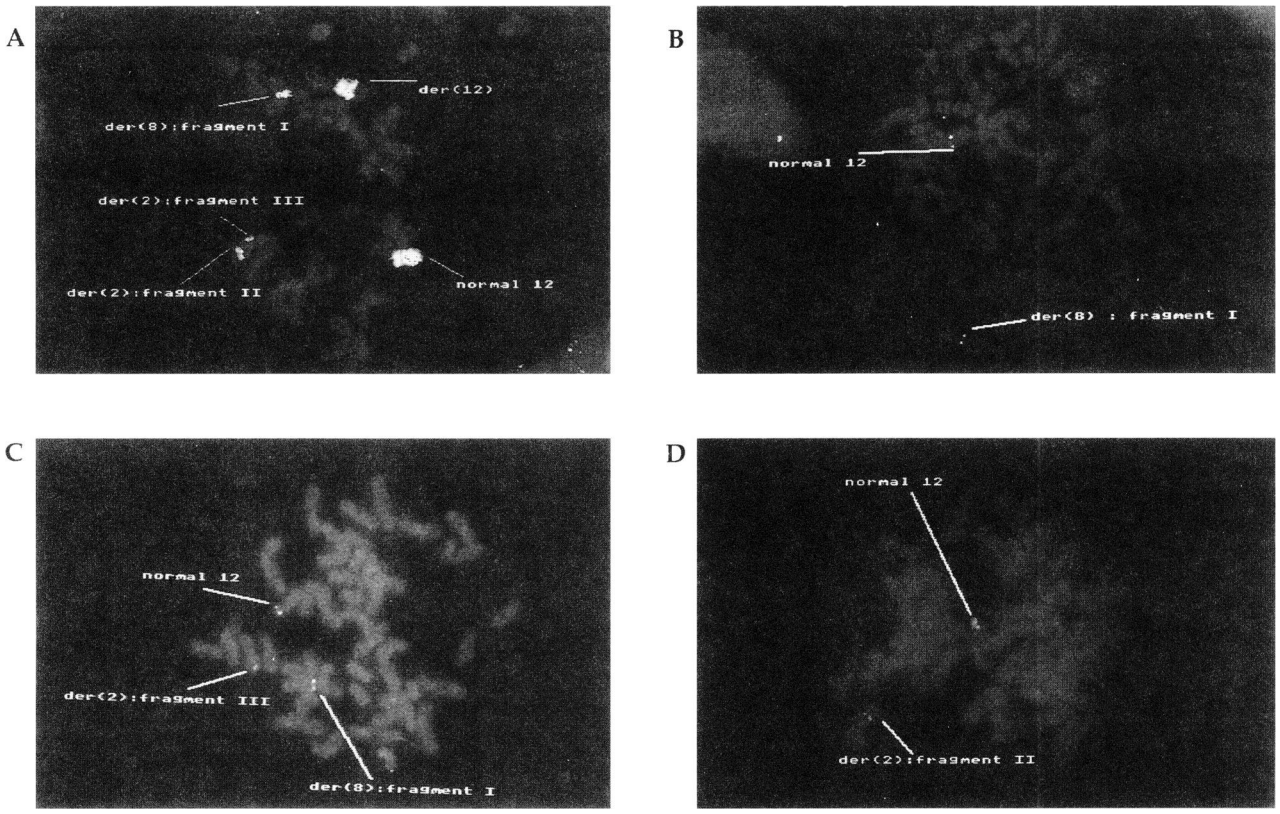

Figure 1 FISH studies on metaphase chromosomes from patient A. *A*, Chromosome 12 paint, which produces five labeled elements. This includes the normal chromosome 12, which is fully labeled, the der (12) chromosome, which shows labeling from 12pter to 12q15 with 10q material translocated distal to this, the der (8) chromosome, which shows interstitial chromosome 12 material at 8pcen (fragment I in fig. 3), and the metacentric der (2) chromosome, which shows 12q material on each arm (telomeric on one arm (fragment III), interstitial on the other arm (fragment II)). *B*, Cosmid 747-A5, which produces positive signals on the normal chromosome 12q and on an interstitial portion of the der (8) chromosome (fragment I). The chromosome 2 material that has been translocated onto the der (8) chromosome makes the der (8p) appear longer than the der (8q). *C*, Simultaneous hybridization with 747-A5 and YAC 804b7, which produces positive signals as in figure 1*B*. In addition the signal from 804b7 is seen on one end of the der (2) chromosome in a telomeric position (fragment III). *D*, Hybridization with YAC 919a2, which produces positive signals on the normal chromosome 12q and on one arm of the der (2) chromosome in an interstitial position (fragment II).

chromosome, is positively labeled by cosmid 747-A5. This cosmid contains STS D12S84, the proximal flanking marker which defines the critical HOS1 region (Bonnet et al. 1994). Thus, breakpoint *x* is proximal to D12S84 and can therefore be excluded from involvement in HOS. Breakpoints *y* and *z*, however, fall within the HOS1 interval. The distal marker flanking HOS1, D12S79 (Bonnet et al. 1994), is present in YAC 804b7. FISH studies demonstrate that 804b7 maps to fragment III on the telomeric end of one of the arms of the der (2) chromosome (fig. 1*C*). This indicates that breakpoint *z* lies proximal to this YAC and therefore also proximal to D12S79. Chromosomal fragment II, which is circumscribed by breaks *y* and *z*, is located on the der (2) chromosome also, but on the opposite arm to fragment III. Figure 1*D* shows that fragment II is labeled positive by YAC 919a2. This YAC contains marker 291xe9, which maps between D12S84 and D12S79. Further YACs, 815f2, and 887b9 and cosmid 14, which maps to the proximal end of YAC 887b9, have been used in FISH studies to determine the limits of fragments I, II, and III. YAC 815f2 maps to fragment I, and 887b9 maps to fragment III. Cosmid 14, however, which is positive for marker 023yd5, a marker present on YAC 887b9, maps to fragment II (data not shown), indicating that breakpoint *z* actually maps within YAC 887b9. These data are summarized in figure 3 and are consistent with the interpretation that chromosome breaks *y* and *z* map between markers D12S84 and D12S79, which flank HOS. Thus, either of these breaks could be responsible for disrupting the HOS gene in patient A.

Discussion

The HOS phenotype is determined early in embryonic development, probably around wk 5 and 6. Cloning and characterization of the HOS1 gene and identification of the mutations underlying this very variable condition

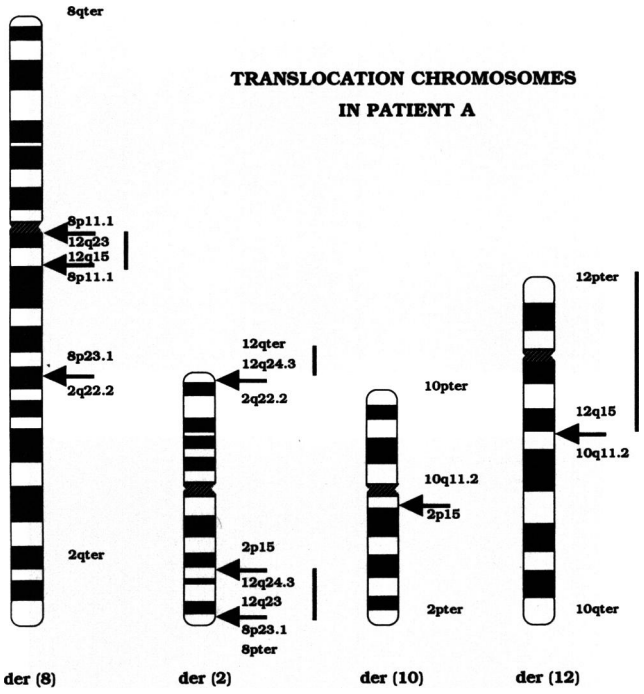

Figure 2 Ideogram, representing the rearranged chromosomes in patient A. The vertical lines on the right of the chromosomes indicate the translocated portions of chromosome 12.

will greatly increase our understanding of heart and limb morphogenesis. It may also point to other genes involved in different heart-hand syndromes if, for example, the HOS1 gene turned out to be a member of a gene family as has been discovered recently for fibroblast growth-factor receptors and craniofacial syndromes (reviewed in Mulvihill 1995). Two other syndromes, ulnar mammary syndrome (Bamshad et al. 1995) and Noonan syndrome (Jamieson et al. 1994) with phenotypes determined early in development map to the same chromosomal region as HOS1. The intervals described for these two syndromes are larger than that for HOS1; nevertheless, the possibility of a developmental gene cluster cannot be excluded.

Translocation breakpoints have proved useful to other positional cloning projects (Ray et al. 1985; Foster et al. 1994; Viskochil et al. 1990), and the lack of further recombination events in our families means that the breaks seen in patient A will greatly speed up the identification of the HOS1 gene. The chromosome 12 painting results have resolved a complex translocation in patient A, indicating that the HOS region at 12q24.1 is rearranged and that two of the breaks fall within the critical HOS1 interval. The locations of these breaks (*y* and *z* in fig. 3) have been determined using FISH with YACs and cosmids from the interval. YAC 919a2 is flanked by breaks *y* and *z*. The position of break *y* is coincidental with a gap in the YAC contig of this region described by Krautner et al. (1995) and Hudson et al. (1995), and this may point to some intrinsic instability in this region. From the FISH data YAC 887b9 appears to map entirely to region III at the telomeric end of the der (2) chromosome, whereas cosmid 14 maps to region II, above breakpoint *z*. Both cosmid 14 and YAC 887b9, however, are positive for marker b023yd5. These data are not necessarily contradictary, since it is likely that YAC 887b9 does not appear to span translocation breakpoint *z* in our experiments because of the representation of the inter-alu PCR product in the hybridization probe or because of the relatively low sensitivity of the FISH. This might indicate that cosmid 14 is close to breakpoint *z*, and as such it may provide a starting point to clone this breakpoint. However, preliminary analysis of genomic

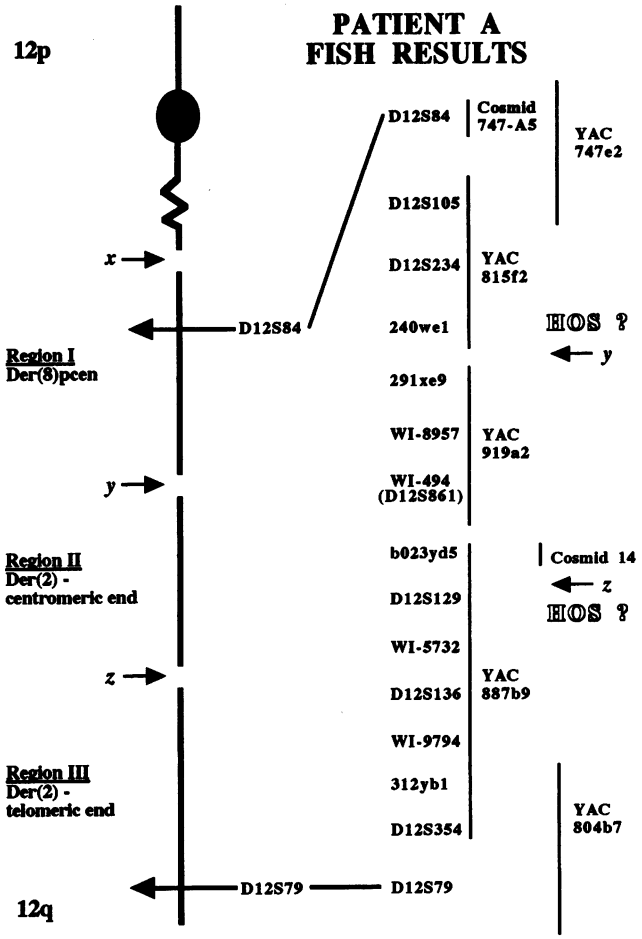

Figure 3 Abridged map of human chromosome 12 (not to scale), highlighting the breakpoints in patient A that are implicated in HOS. The positions of STSs are given on the *left* of the YACs and cosmids containing them in the enlarged part of the figure. The translocated positions of the cosmids and YACs define the breaks: cosmid 747-A5 and YAC 815f2 map to region I, YAC 919a2 maps to region II, and YACs 887b9 and 804b7 map to region III. Thus, the gaps in the contig between 815f2 and 919a2 and the interval distal to b023yd5 on 887b9 correspond to the translocation breakpoints.

DNA from patient A digested with rare cutter enzymes and probed with clones derived from cosmid 14 has, as yet, failed to detect altered restriction fragments (Q. Y. Li, unpublished data).

It is clearly also possible that YACs 815f2 or 919a2 might cross translocation breakpoint *y* and not be detected as spanning clones, because of reasons similar to those outlined above for YAC 887b9. Cosmids derived from the ends of these YACs are currently being characterized to resolve this. In addition, the possibility that the deletion of small amounts of material during the translocation process has caused the HOS phenotype cannot be excluded until the breakpoints are cloned and fully characterized. Thus far, however, no deletions have been identified either by FISH or by testing for loss of microsatellite alleles in patient A.

The chromosomal rearrangement in patient A is complex, and the possibility of genes being disrupted at the other (non-12q) translocation breakpoints cannot be excluded. In such a situation, patient A would represent a HOS phenocopy arising either from the disruption of a single gene at one of the other translocation sites or as a compound phenotype in which components of HOS result from the disruption of more than one gene. We consider each of these two scenarios to be unlikely. Of 23 HOS families reported (Bonnet et al. 1994; Terrett et al. 1994; Basson et al. 1995), 2 are clearly not linked to markers on 12q (Terrett et al. 1994). However, analysis of these two families with markers on chromosomes 2, 8, and 10 (the other chromosomes disrupted in patient A) failed to provide evidence of linkage (J. A. Terrett, unpublished observation). Thus, the likelihood that in patient A the second unmapped HOS gene has been disrupted, rather than the HOS gene on 12q, where two breaks have occurred within the critical 6-cM interval, seems remote. Furthermore, patient A has a classic HOS phenotype (Newbury-Ecob et al. 1996), and it would seem improbable that such a phenotype could be recreated by the disruption of multiple developmentally expressed genes, without producing a range of additional features.

The identification of a HOS patient with a chromosomal rearrangement provides a focus for the HOS gene search. We are currently characterizing clones both adjacent to and spanning the translocation breakpoints on 12q and analyzing them for transcripts in order to identify HOS1 candidate genes. The recently published Whitehead Institute map (Hudson et al. 1995) shows that there are nine ESTs mapping between D12S84 and D12S79. Each of these ESTs—NIB1630, WI-9038, NIB1804, WI-12421, WI-11137, WI-8957, WI-12136, WI-6962, and WI-9426—provides a HOS1 candidate gene. Fine mapping of these cDNAs with respect to the translocation breakpoints in patient A is an immediate priority.

Acknowledgments

We thank the family of the patient described in this report and her obstetricians (Prof. Nicholas Fisk and Mr. D. Rosenberg). We are grateful to Drs. Tony Parkin and Marina Dominguez-Steglich, for help with the FISH and cytogentic analyses, Ms. Susan Blunt, who carried out the original cytogenetic studies, and Professor Ian Young, for comments on the manuscript. YACs were provided by the Human Genome Mapping Project at the Sanger Centre, Cambridge. This work was funded by grants from the British Heart Foundation and the Black Rock Trust.

References

Bamshad M, Krakowiak PA, Watkins WS, Root S, Carey JC, Jorde LB (1995) A gene for ulnar-mammary syndrome maps to 12q23-q24.1. Hum Mol Genet 4:1973–1977

Birnboim HC, Doly J (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 7:1513

Basson CT, Solomon SD, Weissman B, MacRae CA, Poznanski AK, Prieto F, Ruiz de la Fuente WE, et al (1995) Genetic heterogeneity of heart-hand syndromes. Circulation 91: 1326–1329

Bonnet D, Pelet A, Legeai-Mallet L, Sidi D, Mathieu M, Parent P, Plauchu H, et al (1994) A gene for Holt-Oram syndrome maps to the distal long arm of chromosome 12. Nat Genet 6:405–408

Driesen MS, Dauwerse JG, Wapenaar MC, Meershoek EJ, Mollevanger P, Chen KL, Fischbeck KH, et al (1991) Generation and fluorescent in situ hybridization mapping of yeast artificial chromosomes of 1p, 17p, 17q, and 19q from a hybrid cell-line by high-density screening of an amplified library. Genomics 11:1079–1087

Foster JW, Dominguez-Steglich MA, Guioli S, Kwok C, Weller PA, Stevanovic M, Weissenbach J, et al (1994) Campomelic dysplasia and autosomal sex reversal caused by mutation in an SRY-related gene. Nature 372:525–530

Holt M, Oram S (1960) Familial heart disease with skeletal malformations. Br Heart J 22:236–242

Hoffman CS, Winston F (1987) A 10-minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia-coli*. Gene 57:267–272

Hudson TJ, Stein LD, Gerety SS, Ma J, Castle AB, Silva J, Slonim DK, et al (1995) An STS-based map of the human genome. Science 270:1945–1954

Hurst JA, Hall CM, Baraitser M (1991) The Holt-Oram syndrome. J Med Genet 28:406–410

Jamieson CR, Vanderburgt T, Brady AF, Vanreen M, Elsawi MM, Hol F, Jeffery S, et al (1994) Mapping a gene for Noonan-syndrome to the long arm of chromosome 12. Nat Genet 8:357–360

Krautner K, Montgomery K, Yoon SJ, LeBlanc-Straceski J, Renault B, Marondel I, Herdman V, et al (1995) A second generation YAC contig map of human chromosome 12. Nature Suppl 377:321–333

Mulvihill JJ (1995) Craniofacial syndromes: no such thing as a single gene disease. Nat Genet 9:101–102