Am. J. Hum. Genet. 48:696–703, 1991

Human Elastin Gene: New Evidence for Localization to the Long Arm of Chromosome 7

Michael J. Fazio,* Marie-Genevieve Mattei,† Edith Passage,† Mon-Li Chu,* Donald Black,‡ Ellen Solomon,‡ Jeffrey M. Davidson,§ and Jouni Uitto*

*Departments of Dermatology, Biochemistry, and Molecular Biology, Jefferson Medical College, and Section of Molecular Dermatology, Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia; †Centre de Génétique Médicale, INSERM U.242, Marseilles; ‡Imperial Cancer Research Fund, London; and §Department of Pathology, Vanderbilt University and the Veterans Administration Medical Center, Nashville

Summary

In this study we have utilized human elastin cDNAs in molecular hybridizations to establish the chromosomal location of the human elastin gene. First, in situ hybridizations were performed with metaphase chromosomes from phytohemagglutinin-stimulated human peripheral blood lymphocytes. In three separate experiments using two different regions of human elastin cDNAs, the distribution of grains was found to be concentrated on the long arm of chromosome 7 within the [q11.1-21.1] region, and the peak number of grains coincided with the locus 7q11.2. Second, hybridizations with a panel of human-rodent cell hybrids showed concordance with human chromosome 7. Third, PCR analyses with elastin-specific primers of DNA from a hybrid cell line containing chromosome 7 as the only human chromosome yielded a product of the expected size, while DNA containing human chromosome 2, but not chromosome 7, did not result in a product. The results indicate that the human elastin gene is located in the proximal region of the long arm of chromosome 7. The precise localization of the elastin gene in the human genome is useful in establishing genetic linkage between inheritance of an allele with a mutated elastin gene and a heritable disorder.

Introduction

Elastic fibers form a connective-tissue network that is responsible for elasticity and resilience of tissues, such as the lungs, large blood vessels, and the dermis (Rosenbloom 1984; Uitto et al., in press). The predominant component of these fibers is elastin, an extracellular matrix protein synthesized as a precursor, tropoelastin, by a variety of cells, including human skin fibroblasts (Sephel et al. 1987; Fazio et al. 1988*a;* Olsen et al. 1988). Recent characterization of human elastin complementary DNAs (cDNAs) has delineated the entire encoded primary sequence of elastin (Indik et al. 1987; Fazio et al. 1988*a,* 1988*b*). The sequence data indicate that the primary translation product of tropoelastin gene is a polypeptide of ~70 kD, encoded by a 3.5-kb mRNA (Fazio et al. 1988*a;* Olsen et al. 1988). The primary mRNA transcript of the human elastin gene undergoes alternative splicing, so that various exons or portions of exons are removed, leading to formation of a heterogenous group of elastin mRNAs that show variability in the primary nucleotide sequences (Indik et al. 1987; Fazio et al. 1988*a,* 1988*b*).

Recombinant-DNA techniques have been utilized to delineate the structure of the entire human elastin gene (Bashir et al. 1989; Kähäri et al. 1990). These studies have demonstrated that elastin gene is ~45 kb in size and contains 34 exons separated by relatively large introns. Consequently, the intron:exon ratio is unusually high, ~19:1 (Bashir et al. 1989).

Previous studies have suggested that the human elastin gene is located on chromosome 2 (Emanuel et al. 1985). These studies utilized a 421-bp cDNA, corresponding to the 3′ untranslated region of human

Received July 24, 1990; revision received November 13, 1990.

Address for correspondence and reprints: Jouni Uitto, M.D., Ph.D., Department of Dermatology, Thomas Jefferson University, 1020 Locust Street, Philadelphia, PA 19107.

0002-9297/91/4804-0008$02.00

elastin mRNA, to hybridize normal metaphase chromosomes, as well as cells carrying a balanced translocation between chromosomes 1 and 2. These authors concluded that the human elastin gene resides in the region q31-qter of chromosome 2. To map the elastin gene more precisely within the human genome, we have now utilized two separate regions of human elastin cDNAs that encompass both translated and untranslated sequences. Our data, based on chromosomal in situ hybridizations, as well as Southern analyses and PCR amplification of a panel of human-rodent cell hybrid DNAs, localize the human elastin gene to the locus 7q11.2 in the human genome.

Material and Methods

Chromosomal In Situ Hybridizations

Peripheral blood lymphocytes were isolated from normal donors and were stimulated with phytohemagglutinin. The lymphocytes were used to prepare metaphase chromosome spreads for in situ hybridizations with ^{3}H-labeled elastin cDNAs with specific activities of 2×10^8 dpm/μg (Mattei et al. 1985, 1988). The lymphocytes were cultured for 72 h, and BrdUrd (60 μg/ml) was added for the final 7 h of culture to ensure the quality of posthybridization chromosomal banding. To avoid any slippage of silver grains during the banding procedure, chromosome spreads were first stained with buffered Giemsa solution, and the metaphases were photographed. R-banding was then performed by fluorochrome-photolysis–Giemsa method, and the metaphases were rephotographed before analysis, according to a method described elsewhere (Mattei et al. 1985).

Three separate in situ hybridizations were performed utilizing two different regions of human elastin cDNAs. In one set of experiments, metaphase chromosomes were hybridized with a 2.5-kb cDNA, cHDE-4, which encompasses exons 12–36 of the coding region and ~0.9 kb of 3′-untranslated sequences (Fazio et al. 1988*b*). In the second set of experiments, under identical conditions, a 1.1-kb *Bam*HI-*Eco*RI fragment of cHDE-1, which contains sequences for exons 1–18 (Fazio et al. 1988*a*), was used for hybridizations.

The metaphase chromosome spreads were denatured by immersion of slides in 70% (v/v) deionized formamide, 2 × SSC, pH 7.0, at 70°C for 2 min, followed by quick dehydration in cold (4°C) ethanol (50%, 75%, and 95%), and were then air-dried. The radiolabeled elastin cDNA, in a concentration of 25 or 100 ng/ml, was placed on denatured chromosome spreads (25 μl) under a coverslip and incubated at 42°C in a 50% formamide, 2 × SSC saturated environment. After 16–18 h, the slides were dipped in 50% formamide, 2 × SSC at 39°C–40°C to remove the coverslip and then were rinsed three times (10 min each) in 50% formamide, 2 × SSC, pH 7.0, at 39°C to remove unbound cDNA. The slides were then washed three times, for 10 min each, in 2 × SSC, pH 7.0, at 39°C and three times, for 10 min each, in 2 × SSC, pH 7.0, at room temperature. The slides were further washed for 1 h in 0.1 × SSC, pH 7.0 at room temperature and for 1 h in 0.1 × SSC, pH 7.0, at 4°C, prior to being dehydrated through an ethanol series and then air-dried. The autoradiographic exposure time was 7–11 d.

Southern Analyses of Human-Rodent Cell Hybrids

A panel of parental human and rodent (mostly hamster) cell lines, as well as of human-rodent cell hybrids, was used for Southern analyses. The cell hybrids were initially characterized by karyotyping, and the DNA used in this study was further analyzed for the presence of TGFA, PGAM2, and EPO, with known chromosomal locations of 2p13, 7p, and 7q21, respectively (Brissenden et al. 1985; Watkins et al. 1986; Edwards et al.1989; Tsui et al. 1989). Ten micrograms of the hybrid cell line DNA and 5 μg of human DNA were digested with several restriction endonucleases (see Results) and were electrophoresed on 0.8% agarose gels in TAE (Tris-acetate, EDTA) buffer. DNA was then transferred to a nylon membrane (Hybond N^+; Amersham) according to a method described by Maniatis et al. (1989). The filters were hybridized with human elastin cDNA, cHDE-4 (Fazio et al. 1988*b*), labeled by random priming method with [^{32}P]dCTP to a specific activity of 10^8 cpm/μg (Maniatis et al. 1989). Hybridizations were carried out overnight in 1 × SSPE (sodium chloride, sodium phosphate, EDTA), 0.5% SDS, 4% polyethyleneglycol 6000 at 65°C. Heparin (50 μg/ml) and total yeast RNA (50 μg/ml) were used as blocking agents, and no dextran sulfate was used in the hybridizations. The filters were washed twice, for 15 min each, in 1 × SSC at room temperature, followed by two 30-min washes in 0.1 × SSC, 0.5% SDS at 65°C. The [^{32}P]cDNA–genomic DNA fragments were visualized by autoradiography through exposure of the filters to Kodak XAR5 film at −80°C.

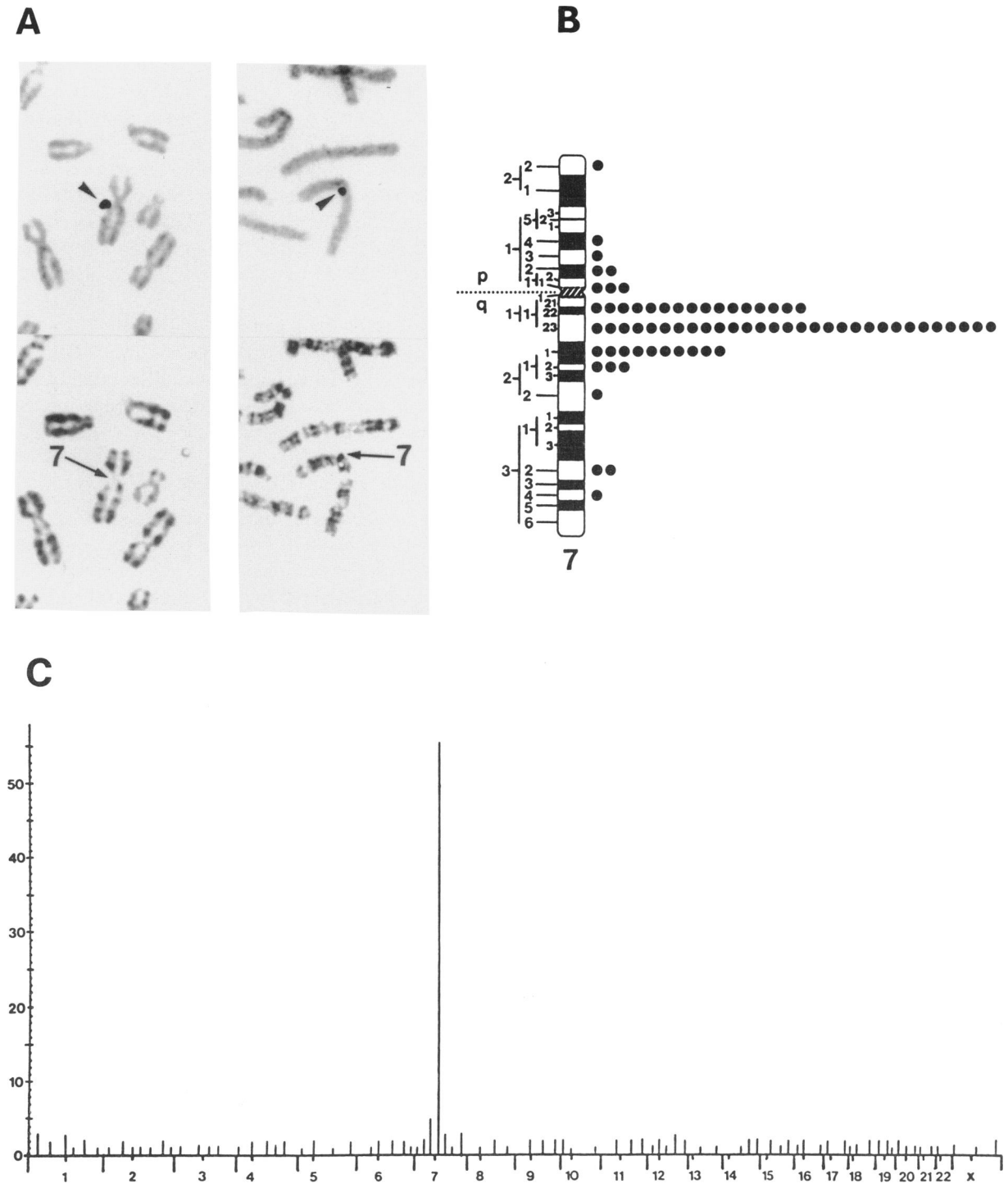

Figure 1 Chromosomal mapping of human elastin gene to locus 7q11.2 by in situ hybridization. *A*, Two partial human metaphases showing specific site of hybridization to chromosome 7. The two top panels indicate the presence of grains representing the ^{3}H-labeled elastin cDNA on Giemsa-stained chromosomes after autoradiography. These chromosomes were subsequently identified as chromosome 7 by R-banding by using the fluorochrome-photolysis–Giemsa method, as shown on the two lower panels. *B*, Idiogram of human G-banded chromosome 7, illustrating distribution of sites labeled by human elastin cDNA. *C*, Histogram depicting grain distribution in 100 metaphases from normal human male DNA hybridized with human elastin cDNA. The abscissa identifies the chromosomes, and the ordinate indicates the number of grains on each chromosome.

Results and Discussion

In the first set of experiments, metaphase cells were examined by chromosomal in situ hybridization with a human elastin cDNA (cHDE-4) in a concentration of 25 ng/ml. Analysis of 100 metaphase cells after an 11-d exposure revealed a total of 189 silver grains associated with chromosomes, with 71 grains (37.5%) being located on chromosome 7 (fig. 1*A–C*). The distribution of grains within chromosome 7 was concentrated in the [q11.1-21.1] region, accounting for 78% of the chromosome 7–specific grains (fig. 1*B*). The peak concentration of grains coincided with band 7q11.2 (fig. 1*B*). A similar experiment with the same probe at a 100 ng/ml concentration, followed by a 7-d autoradiographic exposure, revealed 35% of the grains in association with chromosome 7, with a peak coinciding with the 7q11 region.

The second set of chromosomal in situ hybridization experiments utilized a 1.1-kb 5′-coding fragment of cHDE-1 in a 100 ng/ml concentration (Fazio et al. 1988*a*). The results obtained after a 9-d exposure were similar to those described above, and 21% of the grains were on chromosome 7. Thus, there is strong evidence that the human elastin gene is located in the proximal region of the long arm of chromosome 7 and, more specifically, at the 7q11.2 locus.

Somatic cell hybridization analyses were utilized to confirm the chromosomal in situ hybridization findings. Genomic DNA from human and rodent parent cells, as well as from hybrid cell lines, was digested with *Hpa*II, *Sac*I, *Pst*I, *Bam*HI, *Hin*dIII, and *Eco*RI restriction endonucleases. Southern hybridization of the digested human genomic DNA with a human elastin cDNA (cHDE-4) revealed characteristic bands and, in particular, *Eco*RI bands of 9.5 and 8.5 kb, as predicted from the published map of the human elastin gene (Bashir et al. 1989). Under the stringency of hybridization and washing conditions noted in Material and Methods, no cross-hybridization of the human elastin cDNA to rodent genomic DNA was seen (fig. 2).

The chromosome content of each hybrid cell line was originally determined by karyotyping, as well as by protein and DNA markers. Reanalysis of the DNA used in the present study specifically confirmed the presence of human chromosome 2 sequences in cell lines FG10 and Sir77ii by hybridizations with a TGFA probe (Brissenden et al. 1985), while these two lines were devoid of chromosome 7, as judged from hybridization with an EPO probe at locus 7q21 (Watkins et al. 1986) and by PCR with primers corresponding to PGAM2 at locus 7p (Edwards et al. 1989). Furthermore, chromosome 7 sequences were clearly present in cell lines 3W4C15 and Clone 21, while there was no evidence for the presence of human chromosome 2 when the probes were used. One discrepancy between the initial karyotyping and our characterization of the DNA by chromosome 7–specific markers was noted: although cell line Mog34A4 was initially found, by karyotyping, to contain chromosome 7, no signal either with 7p (PGAM2) or 7q (EPO) probes could be detected. It is likely, therefore, that this cell line had lost human chromosome 7.

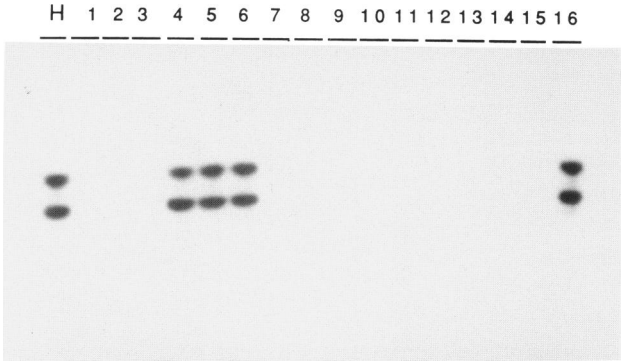

Figure 2 Southern hybridizations of human (H), rodent (lanes 1–3), or human × rodent hybrid cell line (lanes 4–16) DNA digested with *Eco*RI restriction endonuclease. Note that rodent DNA, 10 μg/lane (lane 1, mouse; lane 2, rat; lane 3, hamster), does not hybridize with the human elastin cDNA under the stringency of hybridization and washing conditions used (see Material and Methods). Characteristic bands of ~9.5 and 8.5 kb are readily detected with 5 μg of human DNA (lane H), as well as on lanes 4–6 and 16, which contain 10 μg of DNA isolated from interspecies cell hybrids containing human chromosome 7 (see table 1).

The human elastin gene, as detected by specific bands in Southern analyses (fig. 2), was scored as concordant or discordant with respect to the presence or absence of each human chromosome. These results are presented in table 1. Every chromosome had at least five discordancies (>38% discordancy), with the exception of chromosome 7 (0% discordancy). All hybrids that lacked human chromosome 7 also lacked the elastin gene (table 1). One hybrid (Clone 21), with a human genomic karyotype consisting of only chromosome 7 and being clearly devoid of chromosome 2, was positive in a Southern blot for the elastin gene. Thus, the results of the somatic cell analyses are in agreement with the chromosomal in situ hybridizations, and together these studies provide strong evidence for chromosome 7 localization of the human elastin gene.

As a further approach to mapping the human elastin

Table 1

Segregation of Elastin cDNA with Human Chromosomes in Human × Rodent Hybrid Cell Lines

A. Human Chromosomal DNA

Cell Hybrid	Presence of Human Elastin Gene[a]	Southern[b] Analysis	Presence of Human Chromosomal DNA, by Chromosome																					
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
CTP34B4	+	4	+	+	+	−	+	+	+	+	−	−	−	+	−	+	−	+	+	+	−	−	−	−	+
CTP412A2	+	5	−	+	+	−	−	+	+	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	+
3W4C15	+	6	−	−	−	−	−	−	+	−	−	+	+	+	−	+	+	−	+	−	−	−	+	−	+
FG10	−	7	−	+	−	−	+	−	−	+	−	+	−	−	−	−	+	−	−	+	−	−	+	−	+
DT1.2.4	−	8	−	−	+	−	−	−	−	−	−	−	+	−	−	−	+	−	+	+	−	+	+	+	+
Mog34A4	−	9	+	+	+	+	+	+	−	+	−	+	+	+	+	+	−	+	−	+	+	−	+	−	+
Dur 4.3	−	10	−	−	+	−	+	−	−	−	−	+	+	+	+	+	+	−	+	+	−	+	+	+	+
Horp 9.5	−	11	−	−	−	−	−	−	−	−	−	+	+	+	−	+	−	−	−	−	−	−	−	+	+
Sir77ii	−	12	+	+	+	+	−	−	−	−	−	−	−	+	+	+	−	−	+	−	−	−	+	−	+
Sij4A31[c]	−	13	−	−	+	+	+	+	−	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	+
F4SC13D112	−	14	+	−	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	+
Twin13D12	−	15	+	−	+	+	−	+	−	+	−	−	+	+	−	+	−	+	+	+	−	+	−	+	−
Clone 21	+	16	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

B. Hybrids

Status	No. (%) of Hybrids, by Chromosome																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
Concordant:																							
+ / +	1	2	2	0	1	2	4	1	0	1	1	2	0	3	1	1	3	1	0	0	1	0	3
− / −	5	6	3	5	5	6	9	6	8	5	4	4	6	2	6	6	5	4	8	6	4	5	1
Discordant:																							
+ / −	3	2	2	4	3	2	0	3	4	3	2	2	4	1	3	3	1	3	4	4	3	4	1
− / +	4	3	6	4	4	3	0	3	1	4	6	5	3	7	3	3	4	5	1	3	5	4	8
Total discordant	7 (54)	5 (38)	8 (62)	8 (62)	7 (54)	5 (38)	0	6 (46)	5 (38)	8 (54)	8 (62)	7 (54)	7 (54)	8 (62)	6 (46)	6 (46)	5 (38)	8 (62)	5 (38)	7 (54)	8 (62)	8 (62)	9 (69)

NOTE.—DNA was isolated from human × rodent hybrid cell lines (Croce and Koprowski 1974; Myers et al. 1990).

[a] Examined by Southern hybridizations with a human elastin cDNA, cHDE4 (Fazio et al. 1988*b*).

[b] Numbers refer to electrophoretic lanes in Southern blot shown in fig. 2.

[c] Also has trace of human chromosomes 18 and 22.

gene, PCR with human elastin–specific primers was performed (Fazio et al. 1988*a*). A PCR product of the expected size, ~600 bp, was obtained with cell line Clone 21 DNA used as template, while no signal could be detected with hybrid lines FG10, Sir77ii, or Mog34A4 (results not shown).

Previous studies have suggested that the human elastin gene resides on chromosome 2 (Cicila et al. 1984; Emanuel et al. 1985; Kainulainen et al. 1990). The reason for discrepancy between our results and those reported elsewhere are not readily apparent and could have several explanations. First, human elastin may be encoded by multiple genes, and the probes used could have hybridized to different loci. This explanation is somewhat unlikely, however, since analyses of the elastin gene by Southern hybridizations have indicated a copy number of one (Olliver et al. 1987). Also, the primary nucleotide sequences of human cDNAs, synthesized from human placenta, fetal aorta, or skin fibroblast mRNAs, are identical, with the exception of small areas reflecting alternative splicing (Indik et al. 1987; Fazio et al. 1988*a*, 1988*b*). Furthermore, isolation and characterization of human elastin gene fragments from different genomic DNA libraries have not yielded evidence in support of multiple copies of the gene (Bashir et al. 1989; Fazio et al. 1989; Kähäri et al. 1990). Finally, Southern hybridizations of human genomic DNA digested with *Bam*HI, *Eco*RI, *Hin*dIII, or *Pst*I restriction endonucleases and using either a 421-bp cDNA corresponding to the 3′-untranslated region or a 487-bp cDNA corresponding to the coding region have revealed single bands (Indik et al. 1987). The presence of two bands noted in our hybridizations using the 2.5-kb cDNA (cHDE-4) apparently reflects the size of the probe, and, in fact, the detection of ~9.5- and ~8.5-kb fragments can be predicted from the published gene map (Bashir et al. 1989). It should also be noted that the same two bands were detected in hybridizations with DNA from the cell line Clone 21 which contains chromosome 7 as the only human DNA (see fig. 2). Collectively, these observations suggest that the elastin gene exists as a single copy in the haploid human genome.

A second potential explanation for different results could involve the fact that we utilized human elastin cDNAs different from that used by Emanuel et al. (1985). In the first set of experiments, we used a human elastin cDNA (cHDE-4) which is 2.5 kb in size and encompasses exons 12–36 and 0.9 kb of 3′-untranslated sequences. This probe contains the region of untranslated sequences used by Emanuel et al. (1985) in their studies. In the second set of experiments, we used a 1.1-kb cDNA corresponding to exons 1–18 and devoid of any untranslated sequences (Fazio et al. 1988*a*). In experiments with either one of the cDNAs, 21%–38% of the total grains were found on chromosome 7. In the study by Emanuel et al. (1985), a 421-bp cDNA (pcHEL-1) corresponding exclusively to the 3′-untranslated sequences of the human elastin mRNA was utilized. Since this fragment does not contain elastin-coding sequences, it is possible that this probe could have hybridized to sequences other than those encoding tropoelastin. In this context it is of interest to note that in the rat an ~200-bp segment in the 3′-untranslated region of lysyl oxidase mRNA exhibits a remarkably high homology to a corresponding region in rat as well as human tropoelastin mRNA (Trackman et al. 1990).

The conclusion by Emanuel et al. (1985) that the elastin gene resides in chromosome 2 was also suggested by their observations on a fibroblast strain carrying a balanced translocation between chromosomes 1 and 2. As indicated by Emanuel et al. (1985), the observations made in their studies by chromosomal in situ hybridization of normal metaphase cells and those containing the translocation were internally conflicting, and, specifically, the site of translocation in chromosome 2 was distal to the elastin gene location proposed by chromosomal in situ hybridizations (Emanuel et al. 1985).

Finally, a recent study by Kainulainen et al. (1990), which provided evidence to exclude linkage between the Marfan syndrome and five connective-tissue genes, suggested that the elastin gene may reside in chromosome 2. However, careful examination of their data indicates that the elastin locus was placed ~40 cM away from the nearest marker (COL3A1) on chromosome 2, and the lod scores did not prove linkage.

We believe that our results, based on independent chromosomal in situ hybridizations, as well as on Southern and PCR analyses of human-rodent cell hybrid DNA, clearly localize the human elastin gene to chromosome 7. Also, our findings exclude chromosome 2 localization of the human elastin gene. Specifically, analyses of somatic cell hybrids revealed a 38% discordancy between the elastin gene and chromosome 2, and in all chromosomal in situ hybridization experiments performed in the present study, only background hybridization of the elastin cDNA to chromosome 2—or to other chromosomes, with the exception of chromosome 7—was noted (see fig. 1*C*).

Several other genes coding for extracellular matrix

components of the connective tissue have been mapped to chromosome 7. For example, human proα2(I) collagen gene (COL1A2) has been mapped to locus 7q21.3-q22.1 (Junien et al. 1982), and the human laminin B1 chain gene (LAMB1) resides at chromosomal locus 7q22 (Pikkarainen et al. 1987). However, their precise location within chromosome 7 indicates that they are not closely linked to the elastin gene.

In summary, the results of the present study indicate that human elastin gene resides in chromosome 7, at the 7q11.2 locus. The precise chromosomal mapping of this gene provides additional tools to establish a linkage between the inheritance of an allele with a mutated elastin gene and the clinical phenotype of a heritable disease which affects elastic fibers.

Acknowledgments

The authors thank Cathy Abbott and Robert Knowlton for helpful assistance. Charlene Aranda and Debra Pawlicki provided skillful secretarial help. This study was supported in part by the USPHS and NIH grants AR28450, GM37387, and T32AR7561. A preliminary report of this study was presented at the 51st annual meeting of the Society for Investigative Dermatology, Washington, DC, May 2–6, 1990 (abstract is in J Invest Dermatol 94:523, 1990).

References

Bashir MM, Indik Z, Yeh H, Ornstein-Goldstein N, Rosenbloom JC, Abrams W, Fazio M, et al (1989) Characterization of the complete human elastin gene: delineation of unusual features in the 5′ flanking region. J Biol Chem 269:8887–8891

Brissenden JE, Derynck R, Francke U (1985) Mapping of transforming growth factor alpha gene on human chromosome 2 close to the breakpoint of the Burkitt's lymphoma t (2;8) variant translocation. Cancer Res 45:5593–5597

Cicila G, Yoon K, Ornstein-Goldstein N, Indik ZK, Boyd C, May M, Cannizzaro LA, et al (1984) Elastin gene structure and function. In: Reddi AH (ed) Extracellular matrix: structure and function. Alan R Liss, New York, pp 333–350

Croce CM, Koprowski H (1974) Somatic cell hybrids between mouse peritoneal macrophages and SV-40 transformed human cells. I. Positive control of the transformed phenotype by the human chromosome 7 carrying the SV-40 genome. J Exp Med 140:1221–1229

Edwards HY, Sakoda S, Schon E, Povey S (1989) The gene for human muscle-specific phosphoglycerate mutase, PGAM2, mapped to chromosome 7 by polymerase chain reaction. Genomics 5:948–951

Emanuel BS, Cannizzaro L, Ornstein-Goldstein N, Indik ZK, Yoon K, May M, Oliver L, et al (1985) Chromosomal localization of the human elastin gene. Am J Hum Genet 37:873–882

Fazio MJ, Kähäri V-M, Bashir MM, Saitta B, Rosenbloom J, Uitto J (1989) Regulation of elastin gene expression: evidence for functional promoter activity in the 5′-flanking region of the human gene. J Invest Dermatol 94:191–196

Fazio MJ, Olsen DR, Kauh EA, Baldwin C, Indik Z, Ornstein-Goldstein N, Yeh H, et al (1988*a*) Cloning of full-length elastin cDNAs from a human skin fibroblast recombinant cDNA library: further elucidation of alternative splicing utilizing exon-specific oligonucleotides. J Invest Dermatol 91:458–464

Fazio MJ, Olsen DR, Kuivaniemi H, Chu M-L, Davidson JM, Rosenbloom J, Uitto J (1988*b*) Isolation and characterization of human elastin cDNAs, and age-associated variation in elastin gene expression in cultured skin fibroblasts. Lab Invest 58:270–277

Indik Z, Yeh H, Ornstein-Goldstein N, Sheppard P, Anderson N, Rosenbloom JC, Peltonen L, et al (1987) Alternative splicing of human elastin mRNA indicated by sequence analysis of cloned genomic and complementary DNA. Proc Natl Acad Sci USA 84:5680–5684

Junien C, Weil D, Myers JC, Van Cong N, Chu M-L, Foubert C, Gross M-S, et al (1982) Assignment of the human proα2(I) collagen structural gene (COL1A2) to chromosome 7 by molecular hybridization. Am J Hum Genet 34: 381–387

Kähäri V-M, Fazio MJ, Chen YQ, Bashir MM, Rosenbloom J, Uitto J (1990) Deletion analyses of 5′-flanking region of the human elastin gene: delineation of functional promoter and regulatory *cis*-elements. J Biol Chem 265: 9485–9490

Kainulainen K, Savolainen A, Palotie A, Kaitila I, Rosenbloom J, Peltonen L (1990) Marfan syndrome: exclusion of genetic linkage to five genes coding for connective tissue components in the long arm of chromosome 2. Hum Genet 84:233–236

Maniatis T, Fritsch EF, Sambrook J (1989) Molecular cloning: a laboratory manual, 2d ed. Cold Spring Harbor Laboratory, Cold Spring, Harbor, NY

Mattei MG, Philip N, Passage E, Moisan JP, Mandel JL, Mattei JF (1985) DNA probe localization at 18p11.3 band by *in situ* hybridization and identification of a small supernumerary chromosome. Hum Genet 69:268–271

Mattei MG, Weil D, Prybula-Conway D, Bernard M, Passage E, Van Cong N'G, Timpl R, et al (1988) cDNA cloning, expression and mapping of human laminin B2 gene to chromosome 1q31. Hum Genet 79:235–241

Myers JC, Jones TA, Pohjolainen E-R, Kadri AS, Goddard AD, Sheer D, Solomon E, et al (1990) Molecular cloning of α5(IV) collagen and assignment of the gene to the region of the X chromosome containing the Alport syndrome locus. Am J Hum Genet 46:1024–1033

Olliver L, LuValle PA, Davidson JM, Rosenbloom J, Matthew CG, Beater JA, Boyd CD (1987) The gene coding for tropoelastin is represented as a single copy sequence in the haploid sheep genome. Collagen Relat Res 7:77–89

Olsen DR, Fazio MJ, Shamban AT, Rosenbloom J, Uitto J (1988) Cutis laxa: reduced elastin gene expression in skin fibroblast cultures as determined by hybridizations with a homologous cDNA and an exon-1 specific oligonucleotide. J Biol Chem 263:6465–6467

Pikkarainen T, Eddy R, Fukushima Y, Byers M, Shows T, Pihlajaniemi T, Saraste M, et al (1987) Human laminin B1 chain: a multidomain protein with gene (LAMB1) locus in the q22 region of chromosome 7. J Biol Chem 262: 10454–10462

Rosenbloom J (1984) Biology of disease: elastin: relation of protein and gene structure to disease. Lab Invest 51:605–693

Sephel G, Buckley A, Davidson JM (1987) Developmental initiation of elastin gene expression by human fetal skin fibroblasts. J Invest Dermatol 88:732–735

Trackman PC, Pratt AM, Wolanski A, Tang S-S, Offner GD, Trotler RF, Kagan HM (1990) Cloning of rat lysyl oxidase cDNA: complete codons and predicted amino acid sequence. Biochemistry 29:4863–4870

Tsui L-C, Farrall M, Donnis-Keller H (1989) Report of the Committee on the Genetic Composition of Chromosomes 7 and 8. Human gene mapping 10. Cytogenet Cell Genet 51:166–201

Uitto J, Fazio M, Bashir M, Rosenbloom J. Elastic fibers of the connective tissue. In: Goldsmith L (ed) Biochemistry and physiology of the skin. Oxford University Press, New York (in press)

Watkins PC, Eddy R, Hoffman N, Stanislovitis P, Beck AK, Gall J, Vellucci V, et al (1986) Regional assignment of the erythropoietin gene to human chromosome region 7 pter-q22. Cytogenet Cell Genet 42:214–218