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Linkage of a human brain malformation, familial holoprosencephaly, to chromosome 7 and evidence for genetic heterogeneity

(arhinencephaly/craniofacial development/cyclopia/linkage analysis)

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ABSTRACT **Holoprosencephaly (HPE) is a common malformation of the developing forebrain and midface characterized by incomplete penetrance and variable expressivity. Familial HPE has been reported in many families with autosomal dominant inheritance in some and apparent autosomal recessive inheritance in others. We have examined 125 individuals from nine families with autosomal dominant HPE. Expression in gene carriers varied from alobar HPE and cyclopia through microforms such as microcephaly or single central incisor to normal phenotype. We performed linkage studies by either Southern blot or polymerase chain reaction analyses with DNA markers (*D7S22*, *D7S550*, and *D7S483*) that are deleted from some patients with sporadic HPE and flank a translocation breakpoint in 7q36 associated with HPE. The strongest support for linkage was with *D7S22*, which was linked with no recombination to autosomal dominant HPE in eight of nine families with a combined logarithm of odds score of 6.4 with an affecteds-only model-free analysis and 8.2 with a reduced-penetrance model and all phenotypes. Close linkage to this region could be excluded in one family, and there was significant evidence of genetic heterogeneity. These results show that a gene for autosomal dominant HPE is located in a chromosomal region (7q36) known to be involved in sporadic HPE with visible cytogenetic deletions. They also demonstrate genetic heterogeneity in familial HPE. We hypothesize that mutations of a gene in 7q36, designated *HPE3*, are responsible for both sporadic HPE and a majority of families with autosomal dominant HPE.**

Holoprosencephaly (HPE) is a complex malformation manifested by failure of cleavage of the developing forebrain and associated defects of the midface. It designates a series of brain and face malformations of graded severity that begins with alobar HPE and cyclopia and extends in unbroken sequence through several intermediate forms to a normal brain and face (1, 2). HPE has an estimated incidence of 1 in 16,000 live births and is more common in early gestation with an incidence of 1 in 250 induced abortions (3).

Significant causal heterogeneity has been demonstrated for HPE. It has been associated with several different teratogens such as maternal diabetes (4), although genetic causes are probably more common. It occurs frequently in aneuploidy syndromes, especially trisomies 13 and 18. HPE has been associated also with several nonrandom structural chromosome anomalies including del(2)(p21), dup(3pter), del(7)(q36), del(18p), and del(21)(q22.3). We hypothesized previously that these chromosome regions contain genes important for normal development of the brain and face (2, 5). Alteration of one or several of these putative genes (*HPE1* on chromosome 21q22.3, *HPE2* on 2p21, *HPE3* on 7q36, and *HPE4* on 18p) (6) may lead to the HPE phenotype.

Familial HPE with cytogenetically normal chromosomes has been reported relatively frequently with different families demonstrating autosomal dominant or apparent autosomal recessive patterns of inheritance (5, 7). Some affected individuals from families with the autosomal dominant (AD) form of HPE have had partial expression or microforms of this disorder such as microcephaly, mental retardation, ocular hypotelorism, or single central upper incisor, whereas other obligatory carriers have been phenotypically normal. Despite detailed clinical and neuropathological observations, the molecular basis of HPE and its variable expression remain unknown.

As part of our studies of the genetic causes of HPE, we identified nine families with AD HPE, four of which have been reported (8–11). We hypothesized that the gene or genes involved in familial HPE were the same as those involved with the nonrandom structural chromosome anomalies and selected these regions for study. Here we report linkage of AD HPE in eight of nine families to 7q36.

MATERIALS AND METHODS

Phenotype Evaluation. We identified nine families with AD HPE. Four of these had been reported previously including kindreds 2 (8, 10), 3 (10), 11 (11), and 14 (9). All nine were clinically reevaluated prior to molecular studies. At the time of this reevaluation, informed consent was obtained in accordance with the standards set by local institutional review boards. Family members were classified as having classical HPE, a microform of HPE, or a normal phenotype based on examination by a clinical geneticist (A.L.C., V.P.J., R.C.M.H., G.B.S., M.S.L., C.A.M., W.B.D., or J.C.M., see Fig. 1 and Table 1). Medical records, clinical photographs, and brain imaging studies were reviewed when available. The phenotype evaluations of all involved family members were completed before the molecular studies were initiated.

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Abbreviations: AD, autosomal dominant; HPE, holoprosencephaly; lod, logarithm of odds.

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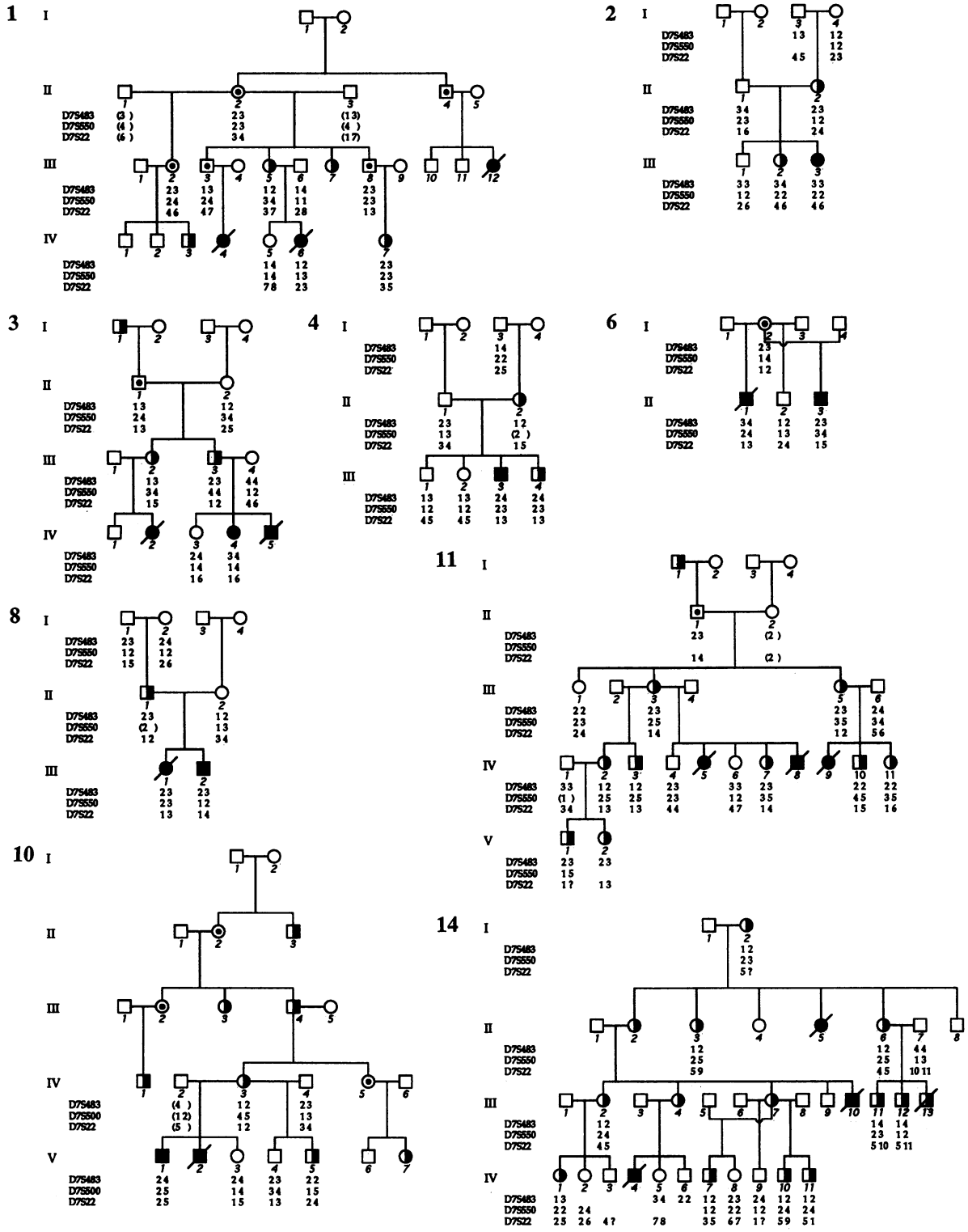

FIG. 1. Pedigrees and genotypes for nine AD HPE families and three 7q36 DNA markers. Individuals with classical HPE have solid symbols, and those with HPE microforms have half-solid symbols. Phenotypically normal obligate carriers are depicted by dotted symbols. The pedigree structures were altered. Several unaffected individuals that were studied at the respective genotypes were omitted to protect confidentiality. Below each symbol are listed pedigree number for each individual in italics and individual alleles for polymorphic DNA markers (*D7S483*, *D7S550*, and *D7S22*) from 7q36. The alleles for PCR markers *D7S483* and *D7S550* were of different size in each family, as was the fragment size at the *D7S22* locus by Southern blot analysis. Alleles shown in parentheses were inferred. Genotype analysis demonstrated linkage between AD HPE and *D7S22* in families 2, 3, 4, 6, 8, 10, 11, and 14, whereas family 1 was not linked.

DNA Analysis. Genomic DNA was isolated from leukocytes or lymphoblastoid cell lines. Southern blot analysis for *D7S22* was done by routine methods (12, 13). Genomic DNA was amplified by polymerase chain reaction (PCR) for microsatellite markers AFM224xh4 (*D7S550*) and AFM074xg5 (*D7S483*) as recommended by the supplier (Research Genetics, Huntsville, AL) (14).

Linkage Analysis. Linkage analyses were completed by using the computer program MLINK (15). An affecteds-only model-free analysis treated unaffected phenotypes as un-

Table 1. Phenotype evaluations in AD HPE families

Kindred	Individual	Age, years	Physical examination	Additional information
1	III.5	39	MC, OH	
	III.7	37	MC, mild OH	
	III.12	0	HPE by report	
	IV.3	?	MO, OC	
	IV.4	0	HPE, cyclopia	
	IV.6	0	HPE, OH	Alobar HPE; hydrocephalus by CT; 46,XX
	IV.7	4	MC, MR	IQ, 45; normal brain MRI
2	II.2	27	SCI, mild OH	
	III.2	4	SCI, OH	
	III.3	2	HPE, MC, severe MR, OH, CLP, PA	Semilobar HPE by CT; 46,XX
3	I.1	50	CL (by report)	
	III.2	30	SCI, OH	
	III.3	28	SCI, OH	
	IV.2	0	HPE, MC, cyclopia	
	IV.4	7	HPE, MC, severe MR, CLP, PA	Lobar HPE by CT; diabetes insipidus; 46,XX
	IV.5	1	HPE, MC, OH, CL, PA	Lobar HPE by CT; 46,XY
4	II.2	26	MC, SCI, short stature	
	III.3	3	HPE, CLP, severe MR	Alobar HPE by US; 46,XY
	III.4	2	MC, CLP, midfacial hypoplasia	Normal MRI of brain
6	II.1	5	HPE, MC, OH, midfacial hypoplasia, severe MR	Semilobar HPE by MRI; estimated IQ, 30; 46,XY
	II.3	0	HPE, MC, OH, CLP	Alobar HPE by MRI; 46,XY
8	II.1	31	Mild MR	Normal brain CT; 46,XY
	III.1	3	HPE, MC, severe MR, spastic tetraplegia	HPE by CT; diabetes insipidus; 46,XX
	III.2	2	HPE, MC, severe MR, spastic tetraplegia	HPE by CT; diabetes insipidus; 46,XY
10	II.3	?	SCI (by report)	
	III.3	?	MC (by report)	
	III.4	?	MC (by report)	
	IV.1	?	CL (by report)	Hydrocephaly
	IV.3	29	CP, SCI, anosmia	
	V.1	12	HPE, MC, OH, severe MR	Alobar HPE by CT; 46,XY
	V.2	0	HPE, MC, OH, single nostril	Alobar HPE by CT and autopsy
	V.5	5	CLP, MC	Normal CT of brain
	V.7	19	MC, OH, SCI	
11	I.1	?	OH	
	III.3	48	Severe MC	
	III.5	35	MC, OH	
	IV.2	25	MC, OH, unusual facies	
	IV.3	23	MC, OH, mild MR, unusual facies	
	IV.5	0	HPE, single nostril, MC	Alobar HPE; absent pituitary on autopsy; 46,XX
	IV.7	14	MO, choanal atresia, OC, MC, SCI, MR	46,XX
	IV.8	0	HPE, extreme OH, MO, single nostril, CLP	Alobar HPE; absent pituitary on autopsy; optic nerve hypoplasia; 46,XY
	IV.9	0	HPE, MO, OH, single nostril	HPE; absent olfactory bulbs and pituitary on autopsy; 46,XX
	IV.10	10	MC, OH, unusual facies	
	IV.11	8	SCI	
	V.1	6	MC, OH, CLP	
	V.2	4	MC, OH	
14	I.2	74	MC	
	II.2	52	MC	
	II.3	53	MC, SCI, OH	
	II.5	0	HPE, MC, CLP, PA	No autopsy available
	II.6	43	MC, OH	
	III.2	33	MC, hyposmia	
	III.4	32	MC, palate anomaly	
	III.7	28	MC	
	III.10	0	HPE, MC, OC, OH, single nostril	Alobar HPE on autopsy
	III.11	22	MC, OH, SCI	Normal brain CT; IQ, 79; 46,XY
	III.12	21	MC, CLP, SCI, mild MR	
	III.13	12	MC, seizures, mild MR	46,XY
	IV.1	13	MC, SCI, mild MR	
	IV.4	1	HPE, MC, MO, OC, OH, PA, seizures	Semilobar HPE by CT; 46,XY
	IV.7	11	MC, OH	
	IV.10	5	MC	
	IV.11	3	MC	

Phenotype information for the nine AD HPE families depicted in Fig. 1. HPE microforms in these families included microcephaly (MC), mental retardation (MR), ocular hypotelorism (OH), microphthalmia (MO), ocular colobomas (OC), cleft lip (CL), cleft lip and palate (CLP), premaxillary agenesis (PA), and single central upper incisor (SCI). Imaging of the brain was done by ultrasonography (US), computer tomography (CT), or magnetic resonance imaging (MRI). Linkage of AD HPE was demonstrated in all families but family 1.

Table 2. Maximum lod scores for chromosome 7q36 markers and familial HPE

Marker	All families			Linked families			Unlinked family		
	Θ	Z	−2 ln L	Θ	Z	−2 ln L	Θ	Z	−2 ln L
				Reduced-penetrance analysis					
D7S483	0.09	2.14	659.6	0.07	1.78	553.6	0.15	0.43	105.6
D7S550	0.04	3.69	657.5[1]	0.00	5.35	520.1	0.44	0.01	129.7
D7S22	0.05	7.03	704.6[2]	0.00	8.19	556.1	0.25	0.46	141.0
				Affecteds-only model-free analysis					
D7S483	0.09	2.05	564.5	0.06	2.17	484.5	0.26	0.12	79.0
D7S550	0.04	3.53	562.7[3]	0.00	4.50	456.6	0.33	0.07	101.3
D7S22	0.06	4.81	619.3[4]	0.00	6.38	497.1	0.33	0.15	114.3

Lod scores (Z) and twice the negative natural logarithm of likelihoods ($-2 \ln L$) are shown for reduced-penetrance and affecteds-only analyses for the full sample, eight linked families, and one unlinked family. Heterogeneity χ^2 values are given for *D7S550* and *D7S22* [1, χ^2 (1) = 7.74, P = 0.005; 2, χ^2 (1) = 7.60, P = 0.006; 3, χ^2 (1) = 4.81, P = 0.028; 4, χ^2 (1) = 7.93; P = 0.005].

known. In a reduced-penetrance analysis, autosomal dominant inheritance of a single gene with penetrance of 67% was assumed, based on segregation analyses in families with AD HPE (7). Both analyses assumed a gene frequency for AD HPE of 1×10^{-6}, which is similar to frequencies of other rare autosomal dominant disorders.

Heterogeneity Test. Because all recombinants between AD HPE and 7q36 markers (*D7S22* and *D7S550*) were observed in one family (kindred 1), a likelihood test (16) was used to evaluate heterogeneity in support of linkage. Twice the difference between the $\log_e$ likelihoods ($-2 \ln L$) for all nine families and the sum of the $\log_e$ likelihoods obtained when the eight linked and one unlinked families were analyzed separately is distributed approximately as χ^2 with 1 degree of freedom.

RESULTS

Phenotype Analyses. We studied nine AD HPE families with three or more affected members or unaffected gene carriers in two to five generations (Fig. 1). Eight kindreds were Caucasian, and none were related. In kindred 6, individual I.2 was Native American. The clinical manifestations in affected family members are summarized in Table 1. Clinical reevaluation of family 11 (11) prior to the genotyping resulted in a reassignment of disease status in individual V.2 who had an HPE microform manifested by microcephaly and ocular hypotelorism. In total, we examined 125 individuals from the nine families including 20 with classical HPE and 40 with HPE microforms.

Linkage Analysis. Genetic analyses were performed in the nine families to determine whether a locus for AD HPE was linked to *HPE3*, one of the proposed genes mapped to 7q36 (12). Polymorphic DNA markers from 7q36 with known map position (7cen–*D7S483*–*D7S550*–*HPE3*–*D7S22*–7qtel) were chosen for the linkage studies (12, 14). The results from the linkage analyses are shown in Tables 2 and 3. The highest logarithm of odds (lod) scores were obtained between AD HPE and *D7S22* (see Table 2). In the reduced-penetrance analysis of the combined sample, a maximum lod score of 7.03 was obtained at a recombination distance of 0.05. There was significant heterogeneity [$\chi^2(1) = 7.60$; $P = 0.005$] with eight families supporting linkage at 0.00 recombination and maximum lod score of 8.19. Close linkage was rejected in family 1 (see Table 3). In the affecteds-only model-free analysis of the combined sample, a maximum lod score of 4.81 was obtained at a recombination distance of 0.06. However, there was again significant heterogeneity [$\chi^2(1) = 7.93$; $P = 0.005$] with eight families (families 2, 3, 4, 6, 8, 10, 11, and 14) supporting linkage at 0.00 recombination and maximum lod score of 6.38. Close linkage was rejected in family 1.

DISCUSSION

Our data show that a gene for familial HPE (AD HPE) is located in chromosome 7q36. AD HPE in eight families maps to this region with a combined lod score of 8.2 and no recombination with *D7S22*. Close linkage between AD HPE and DNA markers (*D7S22*, *D7S550*, or *D7S483*) in 7q36 was excluded in kindred 1 (Fig. 1 and Tables 2 and 3). These results confirm the genetic heterogeneity of familial HPE.

Clinical Manifestations in AD HPE. Clinical evaluation of individuals from the nine families confirmed the previously

Table 3. Lod score table for eight linked and one unlinked family for reduced-penetrance and affecteds-only models

Marker	Recombination fraction (Θ)									
	0.00	0.01	0.02	0.03	0.04	0.05	0.10	0.20	0.30	0.40
					Reduced-penetrance analysis: Eight linked families					
D7S483	0.84	1.30	1.54	1.66	1.73	1.76	1.74	1.31	0.74	0.25
D7S550	5.35	5.23	5.10	4.98	4.86	4.73	4.10	2.80	1.54	0.52
D7S22	8.19	8.03	7.87	7.71	7.55	7.39	6.54	4.76	2.91	1.21
					Affecteds-only model-free analysis: Eight linked families					
D7S483	−0.04	1.65	1.92	2.05	2.12	2.16	2.08	1.47	0.75	0.23
D7S550	4.50	4.39	4.28	4.17	4.06	3.95	3.39	2.27	1.24	0.45
D7S22	6.38	6.24	6.11	5.97	5.83	5.69	4.99	3.55	2.15	0.91
					Reduced-penetrance analysis: One unlinked family					
D7S483	−4.01	−0.37	−0.09	0.06	0.16	0.23	0.40	0.42	0.30	0.15
D7S550	−6.20	−1.84	−1.51	−1.32	−1.17	−1.05	−0.67	−0.28	−0.08	0.00
D7S22	−4.79	−1.66	−1.08	−0.75	−0.53	−0.36	0.12	0.43	0.44	0.28
					Affecteds-only model-free analysis: One unlinked family					
D7S483	−3.96	−0.89	−0.61	−0.45	−0.34	−0.25	−0.03	0.10	0.11	0.07
D7S550	−4.19	−1.11	−0.81	−0.65	−0.53	−0.44	−0.19	0.01	0.07	0.06
D7S22	−3.89	−2.20	−1.62	−1.29	−1.06	−0.88	−0.38	0.02	0.14	0.12

reported phenotypic variability of AD HPE. In each of the nine families, one or more obligate gene carriers had classical (alobar, semilobar, or lobar) HPE, many of whom died during early infancy (Fig. 1 and Table 1). Others had HPE microforms such as microcephaly, mental retardation, microphthalmia, ocular colobomas, ocular hypotelorism, midface hypoplasia, single central upper incisor, cleft lip, and cleft lip and palate. Finally, some obligate gene carriers had normal phenotypes including normal intellect (Fig. 1). The clinical manifestations, including the HPE microforms, did not differ between individuals in the unlinked kindred 1 and the other eight kindreds linked to 7q36 (Table 1). Thus, in AD and sporadic HPE, the phenotype does not appear to differ with alterations of different putative HPE genes.

AD HPE and the *HPE3* Gene. On the physical map of chromosome 7q36, two of the markers used in this study (*D7S22* and *D7S550*) flank a translocation breakpoint that probably disrupts the putative HPE gene, *HPE3*. Previously, we defined the HPE minimal critical region in 7q36 (12). Analysis of 13 HPE cell lines with overlapping deletions involving 7q36 localized *D7S22* to a 5-megabase HPE critical region. A proximal deletion interval was estimated to be 1 megabase in size and contains *D7S550*. The two intervals are separated by the breakpoint of a cytogenetically balanced t(7;9)(q36;q34) translocation (12) that was reported in several individuals with HPE (17). Both the physical map (12) and the linkage map from the present report suggest that the gene that is mutated in most families with AD HPE is the same gene (*HPE3*) that is deleted in some and rearranged in other HPE cell lines with cytogenetic anomalies in 7q36. This hypothesis can be confirmed once the putative *HPE3* gene has been identified by positional cloning of candidate cDNAs from the t(7;9) translocation breakpoint. Because the one 7q36-unlinked AD HPE family is large, it may be possible to identify a second gene locus for familial HPE. Characterization of this and other HPE genes will help to elucidate the molecular basis of both normal and abnormal brain development.

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