The Insulin Gene Is Located on the Short Arm of Chromosome 11 in Humans

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SUMMARY

The human insulin gene has been previously localized to chromosome 11. We have analyzed the human DNA sequences present in a human-mouse somatic cell hybrid line possessing a translocation involving human chromosomes 11 and X. These data indicate that the human insulin gene is located on the short arm of chromosome 11 in the region p13→pter.

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We have recently isolated the human insulin gene and determined its sequence, as well as the general organization of the adjacent DNA regions.[1–4] This gene encodes a 1430-nucleotide insulin messenger RNA precursor that contains two intervening sequences of 179 and 786 nucleotides, that are excised from the precursor to generate the insulin messenger RNA molecule. The insulin messenger RNA directs the synthesis of the insulin precursor protein, pre-proinsulin. This manuscript is concerned with the chromosome localization of the human insulin gene.

Somatic cell hybrids formed between cultured mouse and human cells have been used to map the human genome.[5] These hybrids contain all the mouse chromosomes, but segregate human chromosomes during cell propagation. The mouse and human genes can be discriminated using nucleic acid hybridization techniques, thereby providing a strategy for human gene mapping.[1,6–8] Using these techniques we have localized the human insulin gene to chromosome 11,[1] the growth hormone and chorionic somatomammotropin genes to chromosome 17,[6] the prolactin gene to chromosome 6,[7] and the gene for proopiocortin (ACTH, β-lipotropin) to chromosome 2.[8] We have now analyzed additional human-mouse somatic cell hybrid lines including two that possess a translocation between chromosomes 11 and X. These studies more precisely localize the insulin gene to the short arm of chromosome 11 in the region p13→pter.

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MATERIALS AND METHODS

Cell hybrids. WIL (human WI-38 × mouse LTP), MAR (human GM654 × mouse RAG), DUA (human DUV × mouse A9), EXR (human GM3322 × mouse RAG), and XER (human GM2859 × mouse RAG) were constructed and maintained by methods previously described.[9–11] All human fibroblasts with a GM prefix were obtained from the Human Genetic Mutant Cell repository, Camden, New Jersey. The human parental cell GM2859 possessed a reciprocal translocation [46, X, t (X;11) (Xpter→Xq13::11p13→11pter; Xqter→Xq13::11p13→11qter)] involving chromosomes 11 and X.

Electrophoretic analysis of chromosome 11 enzymes. Lactate dehydrogenase A (LDHA), acid phosphatase-2 (ACP2), and esterase A4 (ESA4) were determined by electrophoretic procedures and histochemical staining as described previously.[9,12] All markers and chromosomes were determined on the same cell passage.

Identification of the human insulin gene. DNA from human, mouse, and human-mouse cell hybrids was isolated and digested to completion with the restriction endonuclease EcoRI. The resulting DNA fragments were separated by electrophoresis on agarose gels and then transferred to nitrocellulose filters.[13] The filters were then hybridized as described previously with an in vitro labeled ^{32}P-labeled human DNA segment prepared from the human insulin genomic clone, λHI-1.[3,4] The human insulin gene is contained within an approximately 14-kilobase (kb) EcoRI-generated DNA fragment.[1,3,4] In contrast, mouse insulin genes are located on three EcoRI-generated DNA fragments of 9.0, 1.8, and 1.4 kb that only weakly cross-hybridize with the human

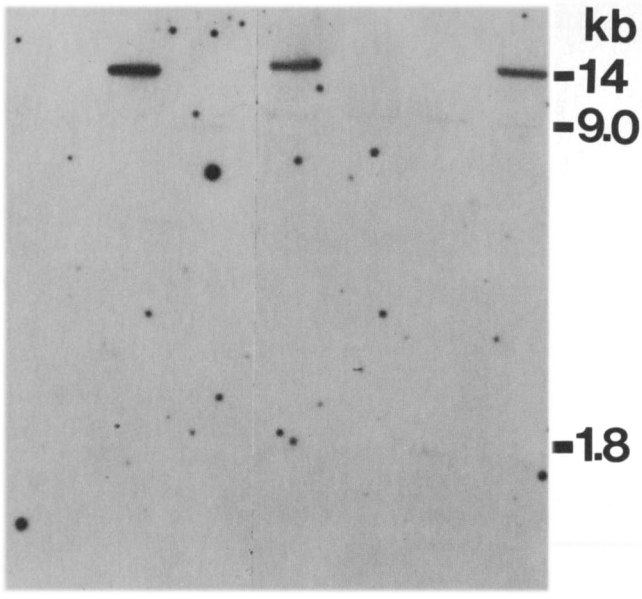

FIGURE 1. Analysis of human and mouse insulin gene containing DNA fragments present in restriction endonuclease digests of human, mouse, and human-mouse cell hybrid DNAs. The hybridization patterns are shown for (A) mouse RAG cells, (B) human T-cell lymphoblasts, (C) hybrid MAR-2, (D) hybrid EXR-5, (E) hybrid XER-7, (F) hybrid XER-9, and (G) hybrid EXR-9. Fragments of HindIII-digested lambda DNA were used as molecular weight markers (New England Biolabs). Channels B, D, and G were positive for the 14-kb fragment containing the human insulin gene. Channels A and C through G were positive for the mouse 9.0-kb and 1.8-kb fragments. These fragments only hybridized weakly with the human insulin gene probe, but were readily evident in the original autoradiograms. The mouse 1.4-kb insulin fragment was not detected in this experiment.

probe under the conditions of hybridization and washing used.[1]

RESULTS

DNA isolated from eight human-mouse cell hybrids was analyzed for the presence of the human 14-kb DNA fragment containing the human gene. The 14-kb DNA segment was present in four of these cell hybrids (Figure 1, Table 1). These cell hybrids were also analyzed for their human chromosome content both by Giemsa-trypsin staining and presence of enzyme markers specific for each of the human chromosomes (Table 1). The 14-kb EcoRI fragment was only present when an intact chromosome 11 was also present (Table 1). These results confirm our previous observations that the insulin gene is located on human chromosome 11.[1]

To determine the regional localization of the human insulin gene (*INS*) on chromosome 11, human cells possessing a reciprocal translocation involving chromosomes 11 and X were fused to mouse RAG cells that were deficient for the enzyme hypoxanthine phosphoribosyl transferase ($HPRT^-$). The resulting hybrids (XER) were isolated using hypoxanthine, aminopterin, thymidine (HAT) selection medium.[14] These hybrids contained a reciprocal translocation between the short arm region of chromosome 11 (11pter→p13) and the long arm region of the X chromosome (Xq13→qter). The chromosomes involved are shown in Figure 2. Since the selectable HPRT marker is located within the q28 region of the X chromosome, the 11/X translocation (Xqter→Xq13::11p13→11qter) must be retained for cell growth on HAT medium. It is therefore possible to regionally map the insulin structural gene. If the insulin gene is present in XER hybrids, it is located in the 11p13→11qter region; if it is absent, it is located in the 11pter→11p13 region.

Two hybrids, XER-7 and XER-9, did not contain a normal intact chromosome 11, but they did contain the 11/X translocation (Figure 2, Table 1). These hybrids did not contain the 14-kb human insulin EcoRI fragment (Figure 1, channels E and F). Three enzyme markers for human chromosome 11 were also tested: lactate dehydrogenase A (LDHA) and acid phosphatase (ACP2), located on the short arm of chromosome 11, and ESA4, located on the long arm of chromosome 11.[15] The regional localizations of these loci are indicated in Figure 2. Hybrids XER-7 and XER-9, missing 11pter→11p13, were negative for LDHA as well as the 14-kb fragment containing the human insulin gene (Table 1), while these hybrids did retain the human chromosome 11 enzyme markers ACP2 and ESA4 and the remainder of chromosome 11 (11p13→11qter). These results are as predicted for a partial chromosome 11 missing the p13→pter region. Thus, the insulin gene is located within the pter→p13 region on the short arm of human chromosome 11.

DISCUSSION

Diabetes mellitus is a family of diseases of diverse origins. A gene near the human lymphocyte D (HLA-D) locus on

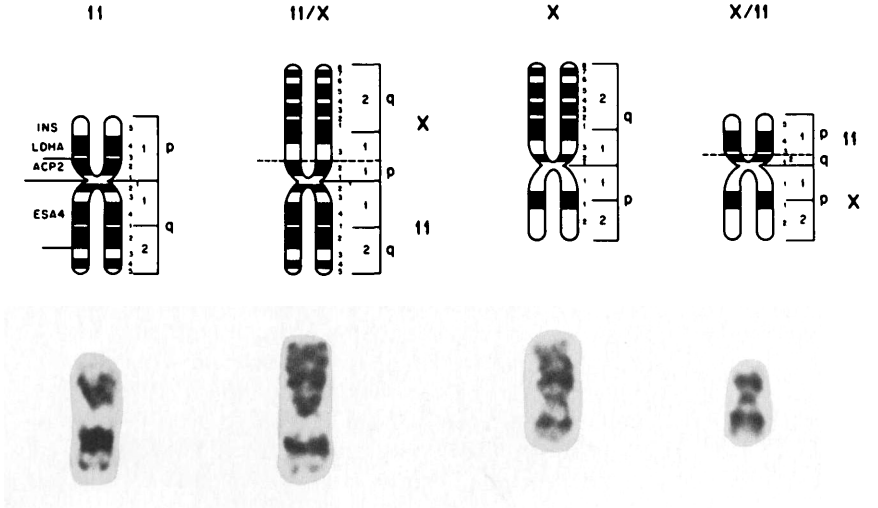

FIGURE 2. Chromosomes involved in the reciprocal translocation. The localization of enzyme markers LDHA, ACP2, and ESA4 has been previously reported.[15]

TABLE 1
Distribution of chromosomes and chromosome 11 gene markers in man-mouse somatic cell hybrids*

Cell hybrids	Chromosome 11 genes				Chromosomes																								
	INS	LDHA	ACP2	ESA4	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	11/X	X/11	X/15	15/X
WIL-3	−	−	−	−	−	−	−	+	−	−	+	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+	+				
WIL-6	+	+	+	+	−	−	−	−	+	+	+	+	−	−	+	−	−	+	−	−	+	−	+	+	+	−	+				
DUA-5†	+	+	+	+	−	−	+	−	+	−	−	−	−	−	+	−	−	+	+	−	+	+	−	−	+	−	−			+	+
MAR-2	−	−	−	−	+	+	−	+	−	−	+	−	−	+	−	−	−	−	+	+	+	−	+	+	+	+	+				
EXR-5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
EXR-9	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
XER-7‡	−	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	−	−	−	−	+	+	−		
XER-9‡	−	−	+	+	−	+	−	+	−	−	−	+	−	+	−	+	−	+	−	−	+	+	−	−	+	−	−	+	−		

* In each cell hybrid, chromosomes and enzymes were determined on the same cell passage. Chromosomes were determined by Giemsa-trypsin staining.[22] Genes coding for enzyme markers representing human chromosomes were tested by isozyme analysis and found to correlate with the human chromosomes retained.
† These hybrids retained translocation chromosomes involving X and 15 derived from the parental human cells.
‡ These hybrids have lost the pter → p13 region of chromosome 11 and do not retain *INS* and *LDHA*. They have retained the 11p13 → qter region of the chromosome involved in the 11/X translocation chromosome and express ACP2 and ESA4.

chromosome 6 may influence the susceptibility to type I, insulin-dependent diabetes.[16] In type II, non-insulin-dependent diabetes, there is a pronounced hereditary predisposition to the disease.[17] The identification of one individual whose diabetes resulted from a mutant insulin with altered biologic properties, as well as the identification of two additional non-diabetes-causing mutant insulins, suggests that certain forms of diabetes may be a consequence of an abnormal insulin molecule encoded by a mutant insulin gene.[18-20] Family studies using polymorphic loci on the short arm of chromosome 11 that are closely linked to insulin (*INS*) may allow us to indirectly determine the prevalence of diabetes caused by mutant insulin genes. The markers to which insulin may be closely linked include NAG (non-alpha globin region), LDHA (lactate dehydrogenase A), Ala1,a3 (lethal antigens a1 and a3), SA11 (surface antigen 1.1), WAGR (Wilm's tumor-aniridia/ambiguous genitalia/mental retardation),[5] and CAT (catalase).[21]

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