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GRANT NUMBER DAMD17-98-1-8305

TITLE: Genomic Imprinting of the M6P/IGF2 Receptor: A Novel Breast Cancer Susceptibility Mechanism

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REPORT DATE: July 1999

TYPE OF REPORT: Annual

PREPARED FOR: Commanding General
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 1999	Annual (1 Jul 98 - 30 Jun 99)

4. TITLE AND SUBTITLE
Genomic Imprinting of the M6P/IGF2 Receptor: A Novel Breast Cancer Susceptibility Mechanism

5. FUNDING NUMBERS
DAMD17-98-1-8305

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
This report contains colored photographs

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The *mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGF2R)* gene encodes for a receptor that plays a critical role in regulating the bioavailability of extracellular proteolytic enzymes and growth factors known to be involved in carcinogenesis. Our recent findings indicate that the *M6P/IGF2R* also functions as a tumor suppressor gene in liver, breast, and lung cancer. We hypothesize that *M6P/IGF2R* gene inactivation, by the novel mechanism of genomic imprinting, results in a non-Mendelian inherited predisposition to breast cancer. We have determined that the frequency of monoallelic *M6P/IGF2R* expression in breast cancer patients is higher than that of age-matched controls and are currently investigating the mechanism underlying the monoallelic expression in these patients. Based on exciting new information we have obtained for Wilms' tumors, we are presently testing the hypothesis that deficiency in *M6P/IGF2R* expression in these breast cancer patients is due to mutations in intron 10 of the *M6P/IGF2R* gene. These results would demonstrate a novel mechanism for the induction of breast carcinogenesis due to inactivation of the *M6P/IGF2R* gene through a post-transcriptional mechanism. Future studies will be directed at determining whether this is a result of spontaneous mutation or rather represents a heritable trait, which may be useful as a predictive and/or prognostic indicator of breast cancer susceptibility.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
43

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

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7/16/99

PI - Signature Date

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INTRODUCTION:

The *mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGF2R)* gene encodes for a receptor that plays a critical role in regulating the bioavailability of extracellular proteolytic enzymes and growth factors known to be involved in carcinogenesis *(1, 2)*. Our recent findings indicate that the *M6P/IGF2R* also functions as a tumor suppressor gene in liver, breast, and lung cancer *(2, 3, 4)*. We have determined that the frequency of monoallelic *M6P/IGF2R* expression in breast cancer patients is higher than that of age-matched controls and are currently investigating the mechanism underlying the monoallelic expression in these patients.

BODY:

Since genomic imprinting leads to uniparental allelic expression, only a single mutation or recombination event would be necessary to functionally inactivate an imprinted tumor suppressor *(2, 5)*. Thus, we have postulated that genomic imprinting of the *M6P/IGF2R* gene would result in a non-Mendelian inherited genetic predisposition to breast cancer. If genomic imprinting of the *M6P/IGF2R* predisposes women to breast cancer, then a significantly higher frequency of *M6P/IGF2R* gene imprinting should exist in breast cancer patients than in age-matched women with no history of cancer.

The initial findings of monoallelic *M6P/IGF2R* gene expression in 2/32 (6%) of the breast cancer patients prompted our investigation into the mechanism directing this phenotype. To eliminate any possibility of partial imprinting interfering with the interpretation of the experimental data, we initially focused our attention on Wilms' tumor patients, which have been documented to have clear and complete monoallelic expression of the *M6P/IGF2R* gene *(6)*. Using four polymorphic markers within the *M6P/IGF2R* gene (mRNA nucleotide positions 901, 1197, 5002, and ACAA, a four-nucleotide insertion/deletion polymorphism in the 3' untranslated region), 2/8 (25%) Wilms' tumor patients were identified which were monoallelic at the cDNA level for the 5002 and ACAA informative loci. Surprisingly, these same two samples were found to have biallelic expression of *M6P/IGF2R* mRNA at the 901 and 1197 informative markers. Therefore, these two Wilms' tumor patients appear to have acquired a mutational event between the upstream (901 and 1197) and downstream (5002 and the ACAA) polymorphic markers which renders one transcript of the *M6P/IGF2R* inactive through truncation.

We have mapped the genomic region in which this mutational event occurred to within intron 10 of the *M6P/IGF2R* gene *(7)*. Intron 10-specific sequence was present in cDNA from one allele of these samples using an intron 10 strand-specific oligonucleotide to prime reverse transcription followed by PCR amplification and nucleotide sequence analysis. It is not clear at this time if this unusual finding can be explained by a previously undefined mechanism which invokes aberrant regulation of genomic imprinting. Alternatively, there may be a mutational event which results in incorrect splicing of intron 10 with loss of downstream message. Similarly, transcriptional termination within intron 10 would produce an RNA transcript from which intron 10

cannot be excised. Current experimental efforts are directed at utilizing 3' RACE (Rapid Amplification of cDNA Ends) to map the 3' site of termination for this RNA. We are also presently in the process of cloning the entire intron 10 (5.5 kb) from both normal and tumor samples to allow for sequencing this intron in its entirety. This will enable us to unequivocally identify the mutation which leads to production of the truncated *M6P/IGF2R* transcript.

Once the mutation(s) within intron 10 is identified, we will return to the samples found to have "monoallelic expression" in breast cancer and evaluate these samples in light of the new information obtained. If these samples are also found to contain the intron 10 mutation, we will re-evaluate all of the breast cancer samples and the normal controls to determine the carriage rate for the intron 10 mutation. It is possible that this mutation may not be able to exert its effect alone, but may require other influential mutations in other genes to effect the production of the truncated RNA. If this is the case, this mutation could represent a predisposing, heritable factor for oncogenesis that might be present in a larger percentage of the population and be of value as a predictive tool for breast cancer susceptibility. In addition, the remaining expressed allele from the breast cancer patients showing "monoallelic expression" will be examined for inactivating mutations which may have directly led to the initiation of breast tumor formation.

KEY RESEARCH ACCOMPLISHMENTS:

- Monoallelic *M6P/IGF2R* 3' end gene expression was found in 2/32 (6.3%) of breast cancer patients, suggestive of either aberrant genomic imprinting and/or a posttranscriptional mechanism which results in the production of a single or truncated mRNA species.

- We have determined that in Wilms' tumor patients, a truncated *M6P/IGF2R* transcript is produced from one allele and have further mapped the site of truncation to within intron 10 of the *M6P/IGF2R* gene.

REPORTABLE OUTCOMES:

Manuscripts:

1. R.L. Jirtle, *Exp. Cell Res.* **248**, 18 (1999).
2. J.G. Falls, D.J. Pulford, A.A. Wylie, R.L. Jirtle, *Am. J. Path.*, **154**, 635 (1999).
3. J.K. Killian, R.L. Jirtle, *Mamm.. Genome*, **10**, 74 (1999).
4. D.J. Pulford, J.G. Falls, J.K. Killian, R.L. Jirtle, *Mutat. Res.*, **436**, 59, (1999).

CONCLUSIONS:

The experimental results generated over the last support period have demonstrated a novel mechanism for production of a functionally monoallelic phenotype in both breast cancer and Wilms' tumor. The mapping of the 3' end of the truncated M6P/IGF2R mRNA in Wilms' tumor to within intron 10 suggests that a mutational event has occurred

within intron 10 which has in some way contributed to oncogenesis in these patients. Mapping of the specific mutation(s) within intron 10 that produce this phenotype will give us information that will be used to re-evaluate both the breast cancer samples and the normal controls to determine the frequency of this genetic alteration and if it is a predisposing factor for breast cancer. If this mutation is found to occur more frequently in the breast cancer samples, it may be useful as a diagnostic/prognostic tool for breast cancer development and survival. Furthermore, this unique mechanism for producing only one functionally active copy of the M6P/IGF2R may predispose individuals to a variety of cancers, and may be useful not only for screening purposes but also in contributing to our understanding of the complex etiology of tumor formation.

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Experimental Cell Research **248,** 18–24 (1999)
Article ID excr.1999.4453, available online at http://www.idealibrary.com on IDEAL®

MINIREVIEW

Genomic Imprinting and Cancer

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Although we inherit two copies of all genes, except those that reside on the sex chromosomes, there is a subset of these genes in which only the paternal or maternal copy is functional. This phenomenon of monoallelic, parent-of-origin expression of genes is termed genomic imprinting. Imprinted genes are normally involved in embryonic growth and behavioral development, but occasionally they also function inappropriately as oncogenes and tumor suppressor genes. The evidence that imprinted genes play a role in carcinogenesis will be discussed in this review. Additional information about imprinted genes can be found on the Genomic Imprinting Website at: (http://www.geneimprint.com).

INTRODUCTION

Genomic imprinting is a non-Mendelian inherited epigenetic form of gene regulation that results in monoallelic expression. In contrast to the random allele inactivation that occurs for example at the *Xist* locus [1], the expressed allele for imprinted genes is dependent upon parental inheritance [for reviews see 2–4]. Thus, genomic imprinting is a phenomenon where the expression of a gene in this generation is dependent upon whether it resided in a male or female the past generation. Epigenetic events such as DNA methylation at CpG sites control the imprinting of genes [4]. Therefore, factors other than the sex of the parent could even modify the imprint process, thereby resulting in a Lamarkian-like inheritance of acquired traits. Such potential imprint-altering factors could include the parental level of nutrition, stress, and exposure to chemical and physical agents.

The existence of imprinted genes first became apparent when nuclear transplantation experiments demonstrated that diploid androgenotes derived from two male pronuclei, and gynogenotes formed from two female pronuclei failed to develop properly during embryogenesis [5, 6]. Similarly, in humans complete hydatidaform moles which contain only paternal chromosomes produce primarily placental tissue, while dermoid cysts which contain only maternal chromosomes produce primarily embryonic tissue [7, 8]. These findings demonstrated that the mammalian genome contains autosomal genes that are only expressed from either the maternal or paternal allele. There are now more than 20 human imprinted genes identified ranging from growth factors to untranslated RNA, and it is postulated that 100 to 500 imprinted genes may exist [for review see 9].

The first endogenous imprinted gene identified was *Igf2* [10]. In 1991 De Chiara *et al.* [10] discovered that homozygous *Igf2*-null mice were approximately 40% smaller than wild-type mice when they were born, consistent with the known growth effects of Igf2. Importantly, the dwarfing phenotype was also observed in heterozygous mice, but only when the mutated allele was inherited from the father. This demonstrated that the *Igf2* gene is imprinted and expressed only from the paternal allele. *IGF2* is also imprinted in human tissues with the notable exception of the adult liver where expression is biallelic because of promoter switching after birth [11].

The second imprinted gene discovered was the maternally expressed *mannose 6-phosphate/insulin-like growth factor 2 receptor (M6p/Igf2r)* [12]. The *M6p/Igf2r* maps to the Tme locus on mouse chromosome 17 [12], and is the gene responsible for this maternal lethal effect [13]. The *M6p/Igf2r* encodes for a receptor that binds both M6P-containing glycoproteins and Igf2 through independent binding sites [for review see 14]. The primary function of this receptor is the intracellular trafficking of phosphomannosyl glycoproteins from the Golgi apparatus to the lysosomes, and the internalization of Igf2 and other extracellular ligands to the lysosomes for degradation [14]. Igf2 signaling is not mediated by M6p/Igf2r, but rather it occurs principally

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0014-4827/99 $30.00

through the Igf1 receptor and the insulin receptor [15, 16].

Thus, the bioavailability of Igf2 is controlled by a receptor that is also imprinted but expressed only from the maternal allele. The reciprocal imprinting of the *Igf2* and *M6p/Igf2r* genes suggested that the evolution of genomic imprinting may have resulted from a parent–offspring conflict to control fetal growth [17]. This parental "tug-of-war" model postulated by Haig [17] predicts that paternally expressed genes promote prenatal and postnatal growth while maternally expressed genes are growth suppressors. The identification of additional imprinted genes and their function will be required to determine whether this provocative model is adequate to explain the evolutionary pressure that resulted in the creation of genes that are functionally haploid [18].

Imprinted genes are not only important in prenatal [10, 19] and postnatal [20, 21] growth control, but also in behavioral development. People with Angelman syndrome, a congenital disease that evidence suggests is caused by the inactivation of the maternally expressed *UBE3A (ubiquitin protein ligase 3A)* gene, are severely retarded in addition to having ataxia, tremulousness, sleep disorders, seizures, and being hyperactive [for review see 9, 22]. *PEG1/MEST,* a member of the α/β-hydroxylase fold family, is a paternally expressed gene that maps to human chromosome 7q32 [23]. *Peg1/Mest(+/−)*-deficient mice are viable and fertile; however, they exhibit growth retardation and increased lethality [24]. Interestingly, the females that inherit the mutated allele from their fathers also have a decreased reproductive fitness because of an abnormal nurturing behavior. It is presently unknown whether *PEG1/MEST* inactivation has a similar effect on maternal nurturing behavior in humans or what effect gene inactivation has in males. Furthermore, the *M6P/IGF2R* has been identified as the first putative "IQ gene," implicating imprinted genes in the development of cognitive ability [25]. Parent-of-origin inheritance effects suggest that imprinted genes are also a genetic determinant in autism [26], bipolar effective disorder [27, 28], and schizophrenia [29], to name only a few [30]. These results demonstrate that imprinted genes play a prominent role in behavioral genetics.

IMPRINTED ONCOGENES AND TUMOR SUPPRESSOR GENES

Imprinted genes are normally involved in embryonic growth and behavioral development; however, occasionally because of inappropriate expression, they also function as oncogenes and tumor suppressor genes. Loss of heterozygosity (LOH) or uniparental disomy (UPD) at an imprinted locus may result in the deletion of the only functional copy of an imprinted tumor suppressor gene [9, 31]. Alternatively, loss of imprinting (LOI) or UPD at an imprinted locus may result in an increased expression of an imprinted proto-oncogene. Furthermore, mutational inactivation of an imprint control center could cause aberrant expression of multiple imprinted proto-oncogenes and/or tumor suppressor genes since imprinted genes often occur in chromosomal domains [32, 33]. Imprinted genes now implicated in human carcinogenesis include: *IGF2, WT1,* $p57^{KIP2}$, *p73, NOEY2,* and *M6P/IGF2R* [9].

Aberrant genomic imprinting and its role in cancer are best exemplified by studies on Wilms' tumor, a sporadic and familial childhood kidney tumor that arises from metanephric blastemal cells. Direct genetic evidence linking tumorigenesis and aberrant imprinting was identified when 70% of Wilms' tumors were found to have biallelic expression of *IGF2* [34–36], a gene that encodes for a growth factor known to be oncogenic when overexpressed [37, 38]. Inactivation of the reciprocally imprinted *H19* gene was also present in a number of these cases [36] suggesting that LOI at the *IGF2* locus in Wilms' tumor could result from loss of *H19* expression [39, 40]. This postulate is supported by the finding that *H19*-null transgenic mice show biallelic expression of *IGF2* [41]. The coupling of biallelic *IGF2* gene expression with *H19* inactivation is even observed in phenotypically normal kidney tissue surrounding Wilms' tumors [42]. Thus, *H19* inactivation and the biallelic expression of *IGF2* appear to be linked and occur early in tumor development. Deregulation of *IGF2* imprinting has now been shown to occur in over 20 different tumor types, demonstrating its fundamental mechanistic importance in carcinogenesis [9].

Another imprinted gene involved in Wilms' tumor formation is *WT1,* a tumor suppressor located at human chromosome 11p13 [43]. *WT1* is biallelically expressed in the kidney, heart, lung, liver, and intestine, but is expressed largely or exclusively from the maternal allele in fetal brain [44]. It is also imprinted in 40% of preterm placenta [44, 45]. Since the imprint status is not correlated with gestational age of the placenta [45], imprinting of the *WT1* gene in the placenta is a polymorphic trait. Imprinting at the *WT1* locus is also polymorphic in fibroblasts and lymphocytes, but the paternal rather than the maternal allele is expressed [46]. These findings suggest the interesting possibility that polymorphic imprinting of the *WT1* tumor suppressor gene could result in both tissue- and individual-dependent susceptibilities to cancer.

The maternally expressed cyclin-dependent kinase inhibitor, $p57^{KIP2}$, maps to human chromosome 11p15.5 [47, 48]. Approximately 10% of Beckwith–Wiedemann syndrome patients have $p57^{KIP2}$ mutations, but $p57^{KIP2}$

has not been found to be mutated in tumors [49, 50]. The maternal allele of $p57^{KIP2}$ is selectively lost in 85% of lung cancers with 11p15 deletions [51]; however, in Wilms' tumors with maternal loss of $p57^{KIP2}$, the normally silent paternal allele is expressed [52]. This suggests that $p57^{KIP2}$ is not a tumor suppressor, at least in Wilms' tumor. Since the imprinting of $p57^{KIP2}$ is incomplete in humans with paternal expression occurring even in some tissues, the putative tumor suppressor function of $p57^{KIP2}$ needs to be further clarified.

NOEY2 is a recently identified member of the *RAS* superfamily with high homology to both *RAS* and *RAP* [53]. It maps to human chromosome 1p31 and is expressed only from the paternal allele. LOH at this locus is observed in 41% of ovarian and breast cancers, and the paternally expressed allele is preferentially deleted. Furthermore, transfection of *NOEY2* into breast and ovarian tumor cells that normally lack expression suppresses growth. Thus, *NOEY2* appears to be an imprinted tumor suppressor gene whose function is frequently abrogated in ovarian and breast cancers.

p73 is an imprinted, maternally expressed gene that encodes for a protein sharing considerable homology with the tumor suppressor *p53* [54]. It maps to human chromosome 1p36, a region containing a putative neuroblastoma tumor suppressor gene expressed predominantly from the maternal allele. The frequent loss of *p73* in neuroblastomas coupled with the demonstration that its overexpression inhibits growth suggested that *p73* is a tumor suppressor gene [55]. Additional studies with a variety of tumors, however, were unable to demonstrate either preferential loss of the expressed maternal allele or somatic mutations in the remaining allele. These findings suggest that *p73* is not the putative imprinted tumor suppressor present at this chromosomal location [56–62].

Monoallelic expression of *p73* has recently been demonstrated in normal lung and kidney tissue, whereas expression is biallelic in the tumors that develop in these tissues [63, 64]. The high frequency of LOI and imprint switching at the *p73* locus in lung cancer and renal cell carcinomas suggest that *p73* is involved in tumorigenesis through the activation of the silent allele and overexpression of wild-type *p73*. Consequently, *p73* may function as an oncogene rather than as a tumor suppressor gene as originally proposed. It would be ironic if both *p53* and *p73* were initially described to have an oncogenic function opposite to that which it possesses.

The *M6P/IGF2R*, at human chromosome location 6q26, is inactivated in a variety of tumors at the earliest stage of transformation [65–68]. It is mutated in 60% of dysplastic liver lesions and hepatocellular carcinomas (HCCs) of patients with or without hepatitis virus (HV) infection [65, 66, 68, 69]. The *M6P/IGF2R* is also mutated in rat liver tumors induced with the genotoxic agent, diethylnitrosamine [70]. The gene contains a poly-G region that is a common mutational target in colon, gastric and endometrial tumors with mismatch repair deficiencies and microsatellite instability [71–73]. Moreover, the *M6P/IGF2R* is mutated in human gliomas that do not contain mutations in the *transforming growth factor β type II receptor* or *Bax* genes [73], and in 30% of human breast tumors [67]. Thus, the *M6P/IGF2R* has been shown to be frequently mutated in a number of different cancers.

Although gene imprinting is often conserved between mammalian species, the imprint status of the *M6P/IGF2R* in humans and rodents is strikingly different. The *M6p/Igf2r* is imprinted in mice [12] and rats [70], but imprinting at this locus appears to be a polymorphic trait in humans, with most individuals having biallelic expression [74–76]. The existence of individuals with an imprinted *M6P/IGF2R* tumor suppressor suggests that they may have increased susceptibility to tumor development because of aberrant imprint control. This postulate is supported by Xu *et al.* [77] who recently reported partial imprinting of the *M6P/IGF2R* in 50% of Wilms' tumor patients. Furthermore, only 1 hit rather than 2 hits would be required to inactive the tumor suppressor function of the *M6p/Igf2r* in mice. This may in part explain why mice are more sensitive to tumor formation than humans. It also suggests that transgenic mice with biallelic expression of the *M6p/Igf2r* may be better human surrogates for carcinogen risk assessment than those presently employed.

The precise molecular mechanism for genomic imprinting of the *M6P/IGF2R* is not completely defined. Methylation of a CpG-rich region in intron 2 (region 2) of the expressed maternal allele carries the imprint signal for this gene in mice [78, 79], and the imprinting box in this region has also now been identified [80]. This region appears to function as the promoter of an antisense transcript that originates only from the repressed paternal allele. This indicates that a form of expression competition may regulate imprinting of the *M6p/Igf2r* gene in mice [79]. Region 2 of the human *M6P/IGF2R* also contains parent-of-origin methylation, but gene expression is biallelic [81, 82]. Consequently, humans and mice appear to possess an altered ability to "read" the *M6P/IGF2R* imprint marks.

M6P/IGF2R inactivation is an early event in liver carcinogenesis, occurring in the initiation rather than the progression stage of transformation (Fig. 1) [68]. Clonal expansion of normal-appearing, preneoplastic hepatocytes with a single *M6P/IGF2R* allele inactivated often occurs in patients chronically infected with hepatitis virus. These precancerous hepatocytes have an enhanced risk of developing into tumors because

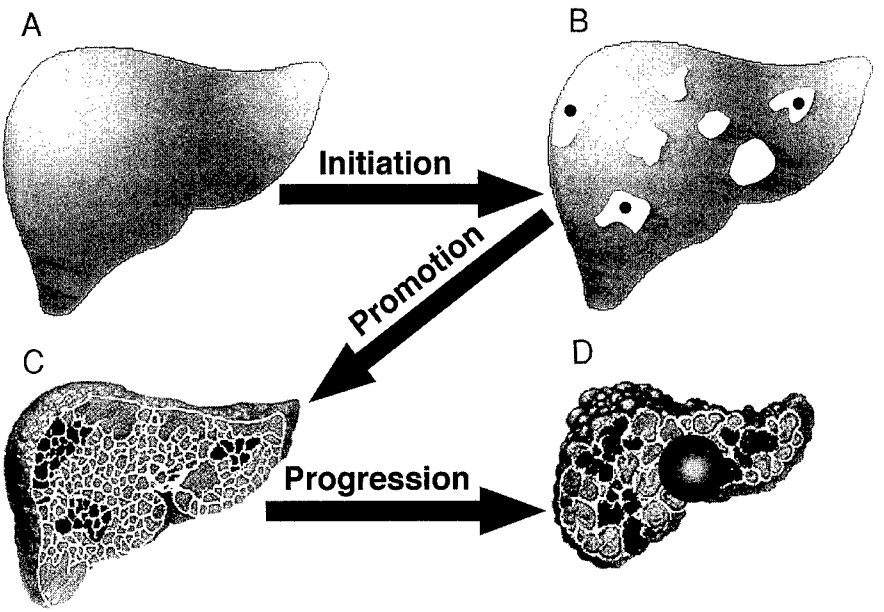

FIG. 1. Oncogenesis model of hepatocellular carcinoma (HCC) development in patients with liver cirrhosis. (A) Normal human liver. (B) Chronic hepatitis virus infection and/or alcohol abuse results in hepatocyte loss (white areas), and the formation of preneoplastic hepatocytes in which a single allele of the *M6P/IGF2R* tumor suppressor gene is inactivated (●). (C) The preneoplastic, *M6P/IGF2R*-mutated hepatocytes preferentially regenerate and/or survive, forming clonal lesions (black areas) in the cirrhotic liver. (D) These clonal regions of preneoplastic hepatocytes (black areas) continue to expand as liver cirrhosis progresses. Approximately 60% of HCCs (large sphere) ultimately arise from this clonally expanded population of preneoplastic, *M6P/IGF2R*-mutated hepatocytes; both alleles of the *M6P/IGF2R* are commonly inactivated in the HCCs.

they ultimately give rise to more than 60% of human HCCs [65, 66, 68]. This suggests that a primary "initiation event" in human liver carcinogenesis is the inactivation of a single allele of the *M6P/IGF2R* gene. The "promotion event" in the transformation process is the clonal expansion of these phenotypically normal, *M6P/IGF2R*-mutated preneoplastic hepatocytes at high risk of completely losing the tumor suppressor function of this gene. All other oncogenic events observed in dysplastic to neoplastic liver lesions occur in the progression stage of transformation. The inactivation of the *M6P/IGF2R* is also an early event in breast cancer [67, 83], but it is unknown whether gene inactivation results in clonal growth in the breast as it does in the liver.

In conclusion, genomic imprinting is an epigenetic form of gene regulation that results in the expression of only one parental allele. Imprinted genes not only play an important role in embryogenesis and behavioral development but are also mechanistically involved in carcinogenesis. Because imprinted genes are functionally haploid, imprinted tumor suppressor genes and proto-oncogenes are particularly vulnerable to inactivation and activation, respectively. The imprinting of genes also varies between species, individuals, tissues, cells, and stage of embryonic development. Therefore, the overall effect of genomic imprinting on cancer susceptibility and penetrance is potentially great.

This study was supported by NIH Grants CA25951 and ES08823, DOD Grant DAMD17-98-1-8305, Rohm & Haas Chemical Company, and Zeneca Pharmaceuticals.

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Received March 3, 1999

American Journal of Pathology, Vol. 154, No. 3, March 1999

Review

Genomic Imprinting: Implications for Human Disease

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Genomic imprinting refers to an epigenetic marking of genes that results in monoallelic expression. This parent-of-origin dependent phenomenon is a notable exception to the laws of Mendelian genetics. Imprinted genes are intricately involved in fetal and behavioral development. Consequently, abnormal expression of these genes results in numerous human genetic disorders including carcinogenesis. This paper reviews genomic imprinting and its role in human disease. Additional information about imprinted genes can be found on the Genomic Imprinting Website at http://www.geneimprint.com. ***(Am J Pathol 1999, 154:635–647)***

Genomic imprinting (also referred to as gametic or parental imprinting) is the epigenetic marking of a gene based on its parental origin that results in monoallelic expression. Genomic imprinting differs from classical genetics in the sense that the parental complement of imprinted genes are not equivalent with respect to their expression, despite both parents contributing equally to the genetic content of their progeny. The mechanism of imprinting is complex and not completely understood; however, evidence suggests that the "imprint mark" is a parental-specific methylation of CpG-rich domains that is established during gametogenesis. The imprint marks on a gene must be erasable in the germline when transmitted through individuals of the opposite sex, but maintained during somatic cell division (Figure 1).

The total number of publications on genomic imprinting has increased markedly over the past 10 years and has now reached almost 1500 (Figure 2). There are now more than 25 identified imprinted genes (Table 1), and estimates based on mouse models indicate that as many as 100 to 200 may exist.[1] Imprinted genes are involved in many aspects of development including fetal and placental growth, cell proliferation, and adult behavior. Consequently, alteration of normal imprinting patterns gives rise to numerous human genetic diseases including cancer. This review examines the role of genomic imprinting in several human genetic diseases such as the Beckwith-Wiedemann, Prader-Willi, and Angelman syndromes, as well as the evidence implicating genomic imprinting in behavioral disorders and carcinogenesis. For excellent reviews on the mechanistic models of genomic imprinting, consult Reik and Walter,[2] Constancia et al[3], and Barlow.[4]

Background

Genomic imprinting plays a critical role in embryogenesis as evidenced by certain aberrations of human pregnancy. The complete hydatidiform mole arises from the fertilization of an anuclear egg either by a haploid sperm (followed by duplication of the paternal genome) or two haploid sperm (diandric diploidy).[5] This trophoblastic disease is characterized by a completely androgenetic (Ag) genome and results in reduced or absent fetal growth coupled with hyperplastic extraembryonic growth.[6,7] In contrast, ovarian dermoid cysts arise from the spontaneous activation of an ovarian oocyte resulting in the duplication of the maternal genome.[8] These abnormalities indicate that normal human development proceeds only when a complete complement of the paternal and maternal genomes is present.

Experimental evidence for the requirement of both the maternal and paternal chromosomal complements was demonstrated through the manipulation of mouse embryos.[9,10] Mouse embryos were altered *in vitro* to produce diploid Ag or diploid parthenogenetic (Pg) embryos, possessing only paternal or maternal chromosomes, respectively. Similarities to the human pregnancy aberrations were apparent since Ag mouse embryos had reduced fetal growth and proliferative extraembryonic growth while Pg embryos maintained relatively normal fetal growth but exhibited poor extraembryonic growth. Nei-

Supported by National Institutes of Health grants CA25951 and ES08823, Department of Defense Grant DAMD17-98-1-8305 (to J.G.F. and R.L.J.), and Zeneca Pharmaceuticals (to D.J.P. and A.A.W).

Accepted for publication January 6, 1999.

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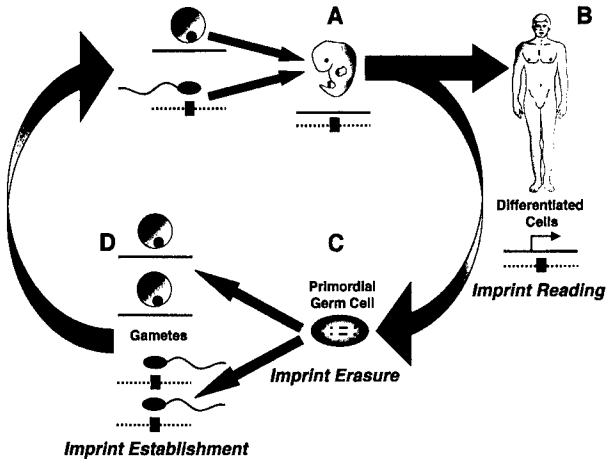

Figure 1. Imprint establishment and propagation during gametogenesis and development. The paternal allele (dashed line) is imprinted and the maternal allele is expressed (solid line). The "imprint mark" (black box) represents a parental-specific methylation established during gametogenesis. A: The maternal and paternal genomes have different imprint patterns following fertilization. B: Both "imprint marks" and imprint reading are maintained during somatic cell division. C: The parental specific imprints are erased in the primordial germ cells. D: The appropriate "imprint marks" are reestablished for the next generation.

ther Ag nor Pg embryos were viable to term.[9,10] This demonstrates that genes expressed exclusively from one parental genome exist, and abnormal embryonic development results from the loss of function of these monoallelically expressed genes. A mark or imprint conferring parental memory must therefore differentiate between the parental genomes, be present on the parental chromosomes through cell division, and be inheritable. This was confirmed when nuclei from early haploid preimplantation embryos were transplanted into fertilized eggs following the removal of one pronucleus. The embryo was viable only if the sex of the donor nucleus was opposite that of the remaining pronucleus.[11]

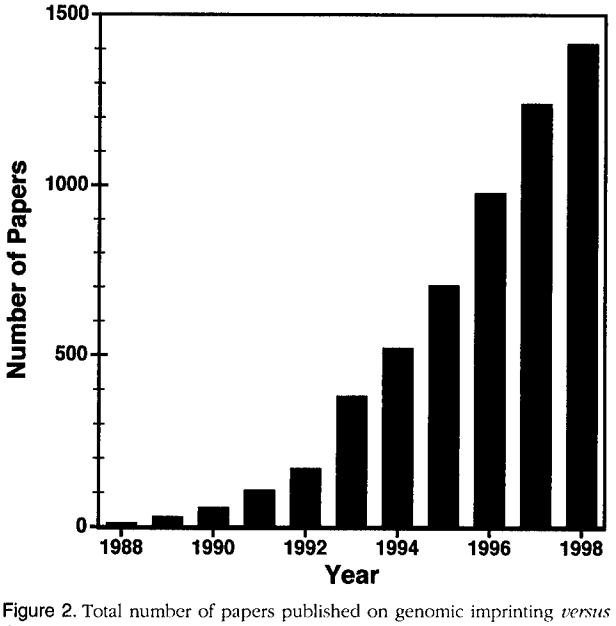

Figure 2. Total number of papers published on genomic imprinting *versus* time.

The chromosomal regions responsible for the genomic imprinting effects observed in mouse embryos were mapped to specific mouse chromosomes by artificially generating uniparental disomies (UPD) in mice. Certain regions of distinct chromosomes were responsible for markedly different phenotypes ranging from embryonic lethality to various growth and developmental defects apparent only after birth. These effects were dependent on whether the two copies were inherited entirely from one parent, resulting in either duplication or deficiency of genes in these chromosomal regions.[12–14] It was initially postulated that only mouse chromosomes 2, 6, 7, 11, 12, and 17 harbored imprinted chromosomal regions.[15] However, there are now reports of other chromosomes either containing more localized areas of genomic imprinting or harboring genes that show more subtle imprinted effects.

UPD also results in phenotypic abnormalities in humans. These include maternal UPD for chromosomes 2, 7, 14, 15, and 16, and paternal UPD for chromosomes 6, 11, 14, 15, and 20.[16] Classic examples of diseases associated with regional maternal and paternal UPD on chromosome 15 include the Prader-Willi syndrome and Angelman syndrome, respectively. Investigations of these genetic diseases are now helping to elucidate the mechanisms of genomic imprinting in humans.

Imprinting of Specific Genes

The first endogenous imprinted gene identified was mouse insulin-like growth factor 2 (*Igf2*), which encodes for a critical fetal-specific growth factor. A targeted mutation in *Igf2* gave rise to a heterozygous dwarfing phenotype when the mutation was passed from the father while the offspring were normal when the mutation was inherited from the mother.[17] Furthermore, the dwarfing phenotype was observed in paternal heterozygotes and homozygotes suggesting that *Igf2* gene expression is exclusively from the paternal allele. At about the same time, the mannose 6-phosphate/insulin-like growth factor type 2 receptor (*M6p/Igf2r*) gene was shown to be imprinted and maternally expressed in mice.[18] Interestingly, the products of these oppositely imprinted genes interact at the biochemical level since the degradation of *Igf2* occurs via the *M6p/Igf2r*.[19] When a mutation was targeted to the *M6p/Igf2r* in mice, maternal heterozygotes or homozygotes showed a 30% increase in fetal growth, but they were not viable at birth.[20] Thus, the reciprocally imprinted *Igf2* and *M6p/Igf2r* genes both play an important role in regulating embryonic development and fetal growth.[17,20]

Numerous techniques have now been used to identify additional imprinted genes. Positional cloning coupled with candidate gene testing has identified novel human imprinted genes located in imprinted clusters at chromosome positions 11p15.5 and 15q11-q13. Techniques have also used parental differences in DNA methylation and expression to identify imprinted genes. Subtractive hybridization or differential display using cDNA from Pg, Ag, and fertilized embryos have yielded novel imprinted

Table 1. Identified Imprinted Genes and Transcripts

Human			Mouse			
Gene	Location	Expressed allele	Gene	Location	Expressed allele	References
NOEY2 (ARHI)	1p31	Paternal				129
p73	1p36	Maternal				147, 148
U2AFBPL	5q22-q31	Biallelic	*U2afbp-rs*	Proximal 11	Paternal	25, 149, 150
MAS1	6q25.3-q26	Biallelic/ Monoallelic in breast	*Mas*	Proximal 17	Paternal	151–153
M6P/IGF2R	6q26-q27	Biallelic/ Maternal*	*M6p/Igf2r*	Proximal 17	Maternal	18, 136–139
			Igf2r-AS	Proximal 17	Paternal	4, 140
GRB10	7p11.2-12	NR	*Meg1/Grb10*	Proximal 11	Maternal	31
PEG1/MEST	7q32	Paternal	*Peg1/Mest*	Proximal 6	Paternal	21, 154, 155
WT1	11p13	Biallelic/ Maternal*	*Wt1*	2	NR	120, 156
ASCL2/HASH2	11p15.5	Maternal	*Mash2*	Distal 7	Maternal	157, 158
H19	11p15.5	Maternal	*H19*	Distal 7	Maternal	30, 159
IGF2	11p15.5	Paternal	*Igf2*	Distal 7	Paternal	17, 36, 160–162
			Igf2-AS	Distal 7	Paternal	36
IMPT1/BWR1A/ ORCTL2/TSSC5	11p15.5	Maternal	*Impt1*	Distal 7	Maternal	163–166
INS	11p15.5	Biallelic	*Ins2*	Distal 7	Paternal	167–169
IPL/TSSC3/BWR1C	11p15.5	Maternal	*Ipl*	Distal 7	Maternal	164, 170, 171
ITM	11p15.5	NR	*Itm*	Distal 7	Maternal	172
KvLQT1	11p15.5	Maternal	*Kvlqt1*	Distal 7	Maternal	62, 173
$p57^{KIP2}$/CDKN1C	11p15.5	Maternal	*$p57^{KIP2}$*	Distal 7	Maternal	48, 122, 174
TAPA1	11p15.5	Biallelic†	*Tapa1*	Distal 7	Maternal?	27, 67, 104
HTR2A	13q14	Biallelic/ Maternal*	*Htr2*	14,Band D3	Maternal	145, 175, 176
FNZ127	15q11-q13	Paternal				177
GABRA5	15q11-q13	Paternal?†	*Gabra5*	Central 7	Biallelic	26, 27, 178
GABRB3	15q11-q13	Paternal?†	*Gabrb3*	Central 7	Biallelic	26, 27, 179
GABRG3	15q11-q13	Paternal?†	*Gabrg3*	Central 7	Biallelic	26, 27, 178
IPW	15q11-q13	Paternal	*Ipw*	Central 7	Paternal	35, 177, 180, 181
NDN (necdin)	15q11-q13	Paternal	*Ndn*	Central 7	Paternal	82, 181, 182
PAR1	15q11-q13	Paternal				177, 180
PAR5	15q11-q13	Paternal				177, 180
PAR-SN	15q11-q13	Paternal				183
SNRPN	15q11-q13	Paternal	*Snrpn*	Central 7	Paternal	84, 184–186
UBE3A	15q11-q13	Maternal	*Ube3a*	Central 7	Maternal	77–79
ZNF127	15q11-q13	Paternal	*Zfp127*	Central 7	Paternal	80, 181, 187
PEG3	19q13.4	Paternal	*Peg3/Apoc2*	Proximal 7	Paternal	22, 188
Neuronatin	20q11.2-q12	NR	*Peg5/Nnat*	Distal 2	Paternal	23, 189, 190
GNAS1	20q13	Paternal	*Gnas1*	Distal 2	Maternal/ Paternal	191–194
XIST	Xq13.2 (XIC)‡	Paternal?	*Xist*	Xic	Paternal	195–200
			$Grf1/Cdc25^{Mm}$	Distal 9	Paternal	24
			Impact	Proximal 18	Paternal	201
			Ins1	Distal 19	Paternal	167, 202

NR, not reported.
* Polymorphic imprinting.
† Determined *in vitro*.
‡ X-inactivation center.

genes such as *Peg1/Mest*, a mesoderm restricted hydrolase at mouse chromosome 6; *Peg3*, a novel zinc-finger protein on proximal mouse chromosome 7; and *Peg5/Nnat* located on mouse chromosome 2.[21–23] The *Grf1* and *U2afl-rs1* imprinted genes were identified by a genome-wide screen termed restriction landmark genome screening (RLGS).[24,25] Finally, three GABAA receptor subunit genes (*GABRB3*, *GABRA5*, and *GABRG3*) were shown to be exclusively expressed from the paternal allele by microcell-mediated chromosome transfer.[26] More recently, results from a somatic-cell hybrid system indicated that these receptor subunit genes were not imprinted.[27]

Characteristics of Imprinted Genes

Several theories have been proposed for the endogenous function of genomic imprinting. Moore and Haig[28] have suggested that genomic imprinting in mammals has evolved from a conflict of interest between the paternal and maternal genome in regulating fetal growth. Whereas

benefits of a large placenta and fetus might ensure future propagation of a paternal line, the result may tax the resources of the mother, thereby compromising future pregnancies. Conversely, if fetal and placental growth is held in check, more offspring from the mother's (and possibly different father's) lineage may be produced. Accordingly, the mother would be predicted to imprint or silence genes that promote placental and fetal growth, whereas the father would imprint genes that inhibit growth.

In support of this theory, the gene encoding the fetal growth factor, *Igf2,* is maternally imprinted, whereas *H19*, which encodes for an untranslated RNA involved in silencing *Igf2* expression, is paternally imprinted.[17,29,30] The result of this reciprocal imprinting is parent-of-origin, monoallelic paternal expression of the gene encoding for Igf2. Interestingly, the genes that encode for the M6p/Igf2r which degrades Igf2, and Meg1/Grb10 which inhibits Igf2 signaling are both paternally imprinted, adding further support for this theory.[18,19,31]

An alternative proposal for imprinting suggests that the cytosine methylation involved in imprint regulation evolved as a defense mechanism for the inactivation of parasitic sequences such as transposable elements and proviral DNA.[32] This is supported by the finding that 5-aza-deoxycytidine, an inhibitor of cytosine DNA methyltransferase, activates silent retroviruses.[33] Irrespective of the reason for the evolution of genomic imprinting in mammals, the functional consequences of genomic imprinting include the inhibition of parthenogenesis and the loss of protection from deleterious recessive mutations.

As more imprinted genes are identified, the characteristics of imprinting are becoming apparent. For example, two chromosomal regions harbor more than one imprinted gene. These imprinting clusters reside at human chromosome 11p15.5 (syntenic to the distal region of mouse chromosome 7) and human chromosome 15q11-q13 (syntenic to the central region of mouse chromosome 7). Within these imprinted gene clusters, genes have been identified that encode for untranslated RNA[34,35] and antisense RNA[36,37] that may be involved in imprint control. Some imprinted genes, such as *H19* and *IGF2,* that are located in imprinted clusters show coordinate regulation. Imprinted genes also often reside in chromosomal regions that undergo asynchronous replication,[38,39] and the meiotic recombination frequencies in these regions may differ between the male and female germ cells.[40] Another characteristic of imprinted genes is an associated allele-specific DNA methylation of cytosine residues in CpG dinucleotides that appears to distinguish the parental alleles.[41–43] Repetitive elements associated with the areas of differential methylation have also been identified in several imprinted genes (ie, *H19*, *M6p/Igf2r*, *U2afbp-rs*, and *p57*^{KIP2}).[44–48]

Imprinting in Genetic Diseases

Beckwith-Wiedemann Syndrome

There are a number of human genetic diseases associated with imprinting defects (reviewed in Refs. 49 and 50). Beckwith-Wiedemann syndrome (BWS) maps to 11p15 and is characterized by general overgrowth with symptoms including hemihypertrophy, macroglossia, and visceromegaly. Genomic imprinting in BWS was first suspected when preferential maternal transmission of mutations was observed in some BWS families.[51] Additionally, approximately 10–20% of BWS individuals are predisposed to embryonal tumors, the most frequent of which are Wilms' tumors and adenocortical carcinoma.[52] The rate of Wilms' tumor formation in the BWS population is 1000-fold higher than in the normal population, and these tumors often show preferential loss of maternal 11p15.[53] The majority of BWS cases arise sporadically; however, in both sporadic and familial forms, a small percentage exhibits UPD at chromosome 11p15. In these cases, the remainder of the chromosome is biparental in inheritance, indicative of somatic mosaicism through a postfertilization mitotic recombination event.[54,55]

The most common molecular event occurring in BWS patients that do not have cytogenetic abnormalities is the biallelic expression of *IGF2* due to loss of imprinting (LOI).[56,57] LOI at the *IGF2* locus may be accompanied by the methylation and/or silencing of the active maternal allele of *H19*.[58,59] This *H19*-dependent event is consistent with an enhancer-competition model for the co-regulation of these genes.[60]

Translocations in BWS patients may also lead to LOI at the *IGF2* locus, but without loss of *H19* imprinting.[61] These translocations affect imprinting by disrupting a gene involved in imprint control, or by altering the function of an imprinting center (IC). Therefore, disruption of *IGF2* imprinting in BWS may also occur via an *H19*-independent event.[56,57] The imprinted *KvLQT1* gene located centromeric to *IGF2* spans a common breakpoint region in BWS, and has been proposed to maintain regional imprint control at 11p15.5.[60] *KvLQT1* shows preferential expression from the maternal allele in most tissues examined except the heart where it is biallelically expressed.[62] This explains why *KvLQT1,* responsible for the autosomal dominant cardiac arrhythmia long QT syndrome, shows no parent-of-origin effect in this disorder. The maternally expressed *p57*^{KIP2}, which encodes for a cyclin-dependent kinase inhibitor, also maps to 11p15.5. Abnormal imprinting and epigenetic silencing of *p57*^{KIP2} is found in some individuals with BWS,[63] and mutations are present in about 5% of BWS patients.[64–66]

To date, ten imprinted genes have been mapped to 11p15.5 (Table 1). Flanking these imprinted genes are the non-imprinted *NAP2* (centromeric border) and *L23MRP* (telomeric border) genes.[67] The syntenic region in the mouse, distal chromosome 7, confirms the existence of an imprinting cluster at this chromosomal location.[68] A possible explanation for the involvement of multiple genes in BWS (even if *IGF2* overexpression is directly responsible for BWS) is that one or more of the adjacent genes (eg, *H19, p57*^{KIP2}, *KvLQT1)* are involved in the regulation of *IGF2* expression. Experimental evidence supports this postulate since transgenic mice that overexpress *Igf2* develop symptoms similar to BWS.[69]

Prader-Willi and Angelman Syndromes

Two clinically distinct genetic diseases associated with genomic imprinting on chromosome 15q11-q13 are the Prader-Willi syndrome (PWS) and the Angelman syndrome (AS). Each syndrome is associated with deficiencies in sexual development and growth, and behavioral and mental problems including retardation.[70,71] Major diagnostic criteria for PWS patients include hypotonia, hyperphagia and obesity, hypogonadism and developmental delay.[72] AS patients often display ataxia, tremulousness, sleep disorders, seizures, and hyperactivity. Severe mental retardation accompanied with a lack of speech may also be present, but AS individuals often display a happy disposition with outbreaks of laughter.[73]

PWS and AS are autosomal dominant disorders showing parent-of-origin effects since the inherited diseases are transmitted from only one of the parents. Approximately 70% of PWS and AS individuals have a *de novo* 3- to 4-megabase deletion in their paternal or maternal chromosome 15q11-q13, respectively. Maternal UPD occurs in most of the remaining PWS patients (25%); however, paternal UPD only occurs in about 4% of AS patients.[16,74] The preferential loss of parental alleles associated with different phenotypes, coupled with the instances of UPD indicate the involvement of imprinted genes (ie, paternally expressed gene(s) for PWS and maternally expressed gene(s) for AS).[70] Recently, approximately 20% of the AS patients without a chromosomal deletion were found to have truncating mutations in *UBE3A*, a gene encoding a ubiquitin protein ligase involved in protein turnover.[75,76] *UBE3A*, mapped to 15q11-q13, has now been reported to be maternally expressed in the human brain.[77,78] Thus, abnormalities in the maternal-specific expression of *UBE3A* during brain development has been proposed for AS.[79] This region also harbors four imprinted, paternally expressed candidate PWS genes: small nuclear riboprotein-associated polypeptide N (*SNRPN*), Imprinted in Prader-Willi (*IPW*), zinc finger 127 (*ZNF127*), and necdin (*NDN*).[35,80–82] The imprinted, paternally expressed transcripts of *PAR1*, *PAR5*, and *PAR-SN* may also be involved in PWS.

Imprinting defects resulting from microdeletions targeted to the *SNRPN* gene have been identified in a small percentage of PWS patients that maintain both parental complements of 15q11-q13.[80,83,84] These deletions alter *SNRPN* promoter methylation and prevent expression of its paternal allele. This results in the silencing of other paternally expressed genes in the cluster.[83,85] These microdeletions apparently disrupt an imprinting center[85] involved in resetting the correct imprinting pattern during gametogenesis.[84,85] The alternate use of *SNRPN* transcripts (BD exons) may be involved in the normal imprinting process.[86] Offspring inheriting microdeletions from their mother exhibit no apparent phenotype; however, a subsequent paternal transmission results in PWS. In comparison, a small percentage of AS patients have similar microdeletions in the *SNRPN* gene (albeit in a region farther upstream) that disrupt the resetting of the imprinting pattern. In this case, progeny inheriting paternal microdeletions do not develop AS, but maternal transmission to offspring results in AS. These PWS and AS microdeletion results support the IC hypothesis, but a bipartite structure must be present since the minimally deleted regions responsible for PWS and AS are distinct.[87] An alternate mechanism for imprinting maintenance in this region relies on an enhancer-competition model between *cis*-linked genes;[4,88] however, methylation analysis of the PWS/AS region reported by Schumacher et al[89] does not support this.

Imprinting in Brain and Behavior Development

The paternally expressed human *MEST* gene maps to 7q32, a region where maternal UPD is associated with intrauterine and postnatal growth retardation.[21,90] Recently, a targeted deletion was introduced into the coding sequence of the mouse *Mest* gene to determine its function.[91] When the deletion was paternally derived, Mest +/− mice were viable and fertile; however, they exhibited growth retardation and increased lethality. *Mest*−/+ animals (deletion maternally derived) showed none of these effects indicating that the phenotypic consequences of this mutation are detected only through paternal inheritance. Interestingly, Lefebvre et al[91] found decreased reproductive fitness in the females that inherited the targeted disruption from their father. This effect was not based on the genotype of the progeny, but rather was due to an abnormal nurturing behavior of the mutant parturient females. Aberrant behavior of the mothers included failure to ingest the extraembryonic tissues (a normal behavior in most mammals), reduced rate of nest building, and pup neglecting. When the pups were fostered to wild-type females, no phenotypic differences between wild-type pups and *Mest*−/+ pups were apparent.

The results of this study demonstrate that the paternally expressed *Mest* is a positive regulator of embryonic growth, and is involved in the regulation of mammalian behavior associated with the rearing of offspring. These findings are consistent with the hypothesis that the imprinting of genes arises from the conflict of interest of the parental genomes in mammals,[28] and supports the importance of imprinted genes in brain development. Previous results using Pg and Ag mouse embryos suggested that both maternally and paternally derived genes contribute to the growth and function of specific brain regions in a complementary fashion.[92] Keverne et al[93] found that Ag cells primarily contributed to hypothalamic composition, whereas Pg cells localized to the cortex, striatum, and hippocampus, but not to the hypothalamus. Brain growth was enhanced by Pg cells and retarded by Ag cells, further supporting the postulate that genomic imprinting is critically involved in mammalian brain development.

Evidence for imprinting effects in human diseases associated with mental abnormalities includes the aforementioned Prader-Willi and Angelman syndromes. There is now also evidence of cognitive imprinting effects in humans displaying normal intelligence. Skuse et al[94] recently reported that an imprinted X-linked locus is poten-

tially responsible for differences in cognitive function of females with Turner's syndrome. Although normal females (46,XX) inherit an X chromosome from both their mother and father, only one X chromosome is inactivated. Turner's syndrome is a sporadic disorder resulting when all or part of one X chromosome is deleted in females. These females display normal intelligence, but overall have a higher incidence of social difficulties.[95,96] Turner syndrome women who inherit the X chromosome from their mother (45,Xm) generally exhibit more behavioral difficulties than those inheriting the X chromosome from their father (45,Xp). This finding provides the first evidence of genomic imprinting on the human X chromosome.[94] Based on cytogenetic analysis of these patients, partial deletions of the short arm of the paternally derived X chromosome were found. This suggests that the putative imprinted locus escapes X-inactivation and potentially lies in Xp11.23-Xqter. Interestingly, Miller and Willard[97] have recently identified a 5.5 megabase region on the human Xp11.21-p11.22 that contains eight expressed sequences which escape X inactivation. However, an imprinted gene(s) in this region is yet to be identified.

Parent-of-origin effects involved in other behavioral and brain disorders have also been reported. Included among these are bipolar affective disorder,[98–100] schizophrenia,[101,102] and autism.[103] However, the involvement of genomic imprinting in these examples remains to be elucidated. For an extensive summary of parent-of-origin effects in human disease, consult Morison and Reeve.[104]

Imprinting in Human Cancer

There are numerous reports of tumors showing a bias in allelic loss. On a genome-wide scale, the complete hydatidiform mole and benign ovarian dermoid cyst arise from cells that are completely Ag or Pg in origin, respectively.[105,106] In addition, numerous tumors are associated with the preferential loss of a particular parental chromosome, indicating the involvement of imprinted genes. Examples include neuroblastoma (maternal chromosome 1p36 and paternal chromosome 2),[107] acute myeloblastic leukemia (paternal chromosome 7),[108] Wilms' tumor (maternal chromosome 11p15.5),[109] rhabdomyosarcoma (maternal chromosome 11p15.5),[110] and sporadic osteosarcoma (maternal chromosome 13).[111] A role for genomic imprinting has also been implicated in the development of familial glomus tumors based on inheritance patterns since tumor susceptibility is inherited paternally.[112]

Imprinted genes can be involved in carcinogenesis in several ways (Figure 3). Loss of heterozygosity or UPD at an imprinted region may result in the deletion of the only functional copy of a tumor suppressor gene. Alternatively, LOI or UPD of an imprinted gene that promoted cell growth may allow gene expression to be inappropriately increased. Finally, mutational inactivation of an IC might result in the aberrant expression of multiple imprinted oncogenes and/or tumor suppressor genes present in an imprinted chromosomal region.

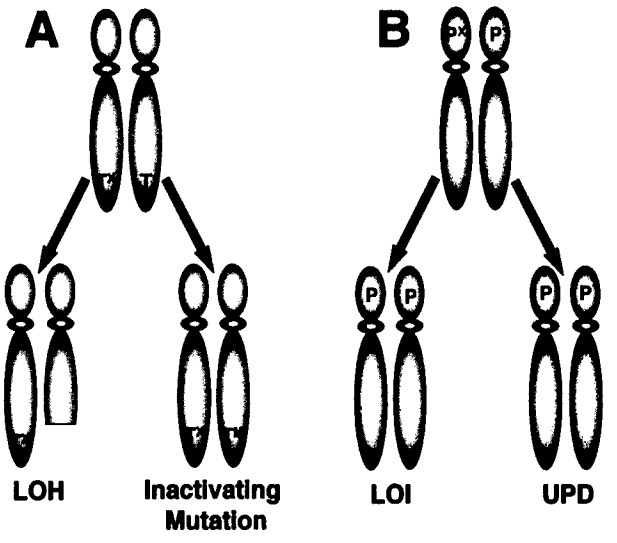

Figure 3. A: Only one allele of a tumor suppressor gene (T) is expressed because of genomic imprinting (T^X). Loss of heterozygosity (LOH) of the expressed allele or an inactivating mutation in the expressed allele (T^M) results in loss of tumor suppressor function. B: Only one allele of the proto-oncogene (P) is expressed because of genomic imprinting (P^X). Loss of imprinting (LOI) or uniparental disomy (UPD) results in biallelic expression of the proto-oncogene.

Aberrant genomic imprinting and its role in cancer are best exemplified by studies on Wilms' tumor, a childhood tumor that arises from metanephric blastemal cells. Direct genetic evidence linking tumorigenesis and aberrant imprinting was identified when 70% of Wilms' tumors were found to have biallelic *IGF2* expression.[113–115] Inactivation of *H19* was also present in a number of these cases.[115] The *H19* gene possesses a CpG island in its promoter that is normally methylated on the paternal allele and unmethylated on the maternal allele.[44,45,115] An enhancer competition model for the reciprocal control of expression of the imprinted *IGF2* and *H19* genes has recently been proposed.[116,117] Thus, LOI of the *IGF2* gene in Wilms' tumor could result from loss of *H19* expression.[116,117] This scenario is supported by the finding that *H19* null transgenic mice show biallelic expression of *IGF2*.[118] The coupling of biallelic *IGF2* gene expression with *H19* inactivation is even observed in phenotypically normal kidney tissue surrounding the Wilms' tumor. This suggests that the inactivation of *H19* and the biallelic expression of *IGF2* are linked, and occur early in development.[119] Other human malignancies showing LOI at the *IGF2* locus are presented in Table 2. These results indicate deregulation of *IGF2* imprinting is mechanistically involved in the development of a variety of tumors.

Because imprinted genes are functionally haploid, an imprinted tumor suppressor gene would be predicted to increase cancer susceptibility since the inactivation of only one allele would eliminate tumor suppressor function. *WT1*,[120,121] $p57^{KIP2}$ [122-124] and *M6P/IGF2R*[125–128] represent imprinted genes implicated in tumor suppression. $p57^{KIP2}$, mapped to 11p15.5, encodes for a cyclin-dependent kinase inhibitor that is maternally expressed. Epigenetic silencing of the expressed allele has been reported in some tumors and BWS patients.[63] Additionally, approximately 5% of BWS patients have $p57^{KIP2}$

Table 2. Aberrant Imprinting in Human Cancer

Tumor type	Gene	Reference
Childhood Tumors		
Wilms' tumor	*IGF2,H19,p57^{KIP2},M6P/IGF2R*	63, 113, 139, 162
Rhabdomyosarcoma	*IGF2*	203
Ewing's sarcoma	*IGF2*	204
Hepatoblastoma	*IGF2*	205, 206
Adult Tumors		
Bladder	*IGF2,H19,IPW*	207, 208
Breast	*IGF2*	209, 210
Cervical	*IGF2,H19*	211
Choriocarcinoma	*IGF2,H19*	212
Colorectal	*IGF2*	213
Esophageal	*H19*	214
Gastric adenocarcinoma	*IGF2*	215
Glioma	*IGF2*	216
Hepatocellular	*IGF2,H19*	217, 218
Leukemia-acute myeloid	*IGF2*	219
Leukemia-chronic myelogenous	*IGF2*	220
Lung	*IGF2,H19,p73*	221–223
Medulloblastoma	*IGF2,H19*	224
Mesothelioma	*IGF2*	225
Ovarian	*IGF2*	226
Prostate	*IGF2*	227
Renal cell carcinoma	*IGF2,p73*	148, 228
Testicular germ cell	*IGF2,H19*	229
Uterine	*IGF2*	230

mutations;[64] however, *p57^{KIP2}* mutations have not been identified in tumors. Thus, the putative tumor suppressor function of *p57^{KIP2}* remains to be clarified. Recently, *NOEY2 (ARHI)*, a novel *ras*-related, maternally imprinted gene at 1p31, was identified as a putative tumor suppressor gene in breast and ovarian carcinomas. In the majority of cases, the functional allele was lost.[129]

Recent reports demonstrate that the *M6P/IGF2R* at 6q26 is inactivated in a variety of tumors at the earliest stage of transformation.[126–128] The *M6P/IGF2R* plays an integral part in the intracellular sorting of lysosomal enzymes, the activation of the growth inhibitor transforming growth factor-β1 (TGF-β1), and the degradation of IGF2, but it is not directly involved in cell signaling.[19,130] The *M6P/IGF2R* is mutated in 60% of dysplastic liver lesions and hepatocellular carcinomas of patients with or without hepatitis virus infection.[125,126,128] The *M6P/IGF2R* is also mutated in 30% of breast tumors,[127] and the gene contains a polyG region that is a common mutational target in colon, gastric and endometrial tumors with mismatch repair deficiencies and microsatellite instability.[128,131,132] Moreover, it has recently been reported that the *M6P/IGF2R* is mutated in human glioma samples that do not contain mutations in the transforming growth factor-β type II receptor (*TGFBRII*) or *Bax* genes.[133] In both breast[127,134] and liver carcinogenesis,[128] the allelic inactivation of *M6P/IGF2R* occurs as an early event, during the initiation rather than the progression stage of transformation.

Although imprinting among individuals and mammalian species is generally conserved, the imprint status of *M6P/IGF2R* in humans and rodents is strikingly different. The *M6p/Igf2r* is imprinted in mice[18] and rats,[135] but imprinting at this locus appears to be a polymorphic trait in humans, with most individuals having biallelic expression.[136–138] The existence of individuals with an imprinted *M6P/IGF2R* tumor suppressor suggests that they may have increased susceptibility to tumor development because of aberrant imprint control. This postulate is supported by Xu et al[139] who recently reported partial imprinting of *M6P/IGF2R* in 50% of Wilms' tumor patients.

The precise molecular mechanism for genomic imprinting of *M6P/IGF2R* is not completely defined. Methylation of a CpG rich region in intron 2 (Region 2) of the expressed maternal allele has been shown to carry the imprint signal for this gene in mice.[46,140] Birger et al[141] have identified a 113-bp sequence, in region 2 of the mouse *M6p/Igf2r* gene, that serves as a methylation imprinting box responsible for the establishment of differential methylation. Furthermore, this region appears to function as the promoter of an antisense transcript that originates only from the repressed paternal allele. This indicates that a form of expression competition regulates imprinting of the *M6p/Igf2r* gene in mice.[140] Region 2 of the human *M6P/IGF2R* also contains parent-of-origin methylation, but gene expression is biallelic.[142,143] Consequently, humans and mice appear to possess an altered ability to read the *M6P/IGF2R* imprint marks.

Functional polymorphic imprinting has also been observed for human genes encoding *IGF2,*[144] *WT1,*[120] and the human 5-HT2A receptor gene *HTR2A.*[145] Recently, the mouse *Kvlqt1* gene has been shown to undergo developmental relaxation of imprinting in a strain-dependent fashion.[146] Whether polymorphic genomic imprinting occurs in other genes, and functions in determining individual and/or species differences in susceptibility to diseases remains to be determined.

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Mammalian Genome 10, 74–77 (1999).

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Genomic structure of the human M6P/IGF2 receptor

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Received: 14 July 1998 / Accepted: 3 September 1998

The human mannose 6-phosphate/insulin-like growth factor 2 receptor (M6P/IGF2R) gene located at 6q26 (Rao et al. 1994) encodes for a multifunctional receptor that possesses distinct binding sites for phosphomannosyl glycoproteins and IGF2 (MacDonald et al. 1988; Morgan et al. 1987), retinoic acid (Kang et al. 1997), and urokinase-type plasminogen activator receptor (uPAR; Nykjaer et al. 1998). The receptor is involved in fetal development, tumor suppression, maternal regulation of intrauterine growth, and has recently been associated with the development of human cognitive ability (Chorney et al. 1998; De Souza et al. 1997; Kornfeld 1992; Wang et al. 1994). To facilitate further genetic analyses of the human M6P/IGF2R, we have determined its complete genomic organization, defined the intron-exon boundary sequences, and designed intronic oligonucleotides for PCR amplification of the 48 exons included in the 136-kb genomic sequence.

The M6P/IGF2R is a chimeric receptor possessing binding sites for four distinct classes of ligands (Kang et al. 1997; Nykjaer et al. 1998; MacDonald et al. 1988; Morgan et al. 1987). The M6P binding site enables the M6P/IGF2R to target to the lysosomes both newly synthesized lysosomal enzymes from the Golgi and phosphorylated proteolytic enzymes endocytosed from the extracellular environment (Kornfeld 1992). Furthermore, the latent complex of transforming growth factor beta (TGFβ), a potent growth inhibitor, binds to the M6P/IGF2R through these M6P binding sites, thereby facilitating its activation by plasmin (Dennis and Rifkin 1991). The effectiveness of TGFβ activation may be further enhanced by the direct binding of uPAR to the M6P/IGF2R (Nykjaer et al. 1998). The mammalian M6P/IGF2R also contains an independent IGF2 binding site that is absent in chickens (Zhou and Sly 1995). IGF2 binding to the M6P/IGF2R leads to lysosomal degradation rather than intracellular signaling, a process mediated by both the IGF1 and the insulin receptors (Kornfeld 1992). Therefore, loss of M6P/IGF2R function would be predicted to increase the extracellular concentration of IGF2, decrease the level of active TGFβ, and increase the secretion of proteolytic enzymes. These biological effects suggest the M6P/IGF2R functions normally as a tumor suppressor.

A number of reports now strongly support this postulate for a wide variety of human tumors. Briefly, the M6P/IGF2R is mutated in 60% of dysplastic liver lesions and HCCs in patients with or without hepatitis virus (HV) infection (De Souza et al. 1995a, 1995b; Yamada et al. 1997). Its inactivation also plays a prominent role in the early stage of breast cancer development (Hankins et al. 1996), and increased expression of the wild-type receptor in breast cancer cell lines leads to apoptosis (Oates et al. 1998). Furthermore, the M6P/IGF2R gene contains a poly-G region that is a common mutational target in colon, gastric, and endometrial tumors with mismatch repair deficiences and microsatellite instability (Souza et al. 1996). Thus, M6P/IGF2R inactivation is a frequent oncogenic event that occurs early in carcinogenesis.

A full parental complement of autosomal genes is inherited by all offspring, but not all are biallelically expressed. The phenomenon of monoallelic expression of the same parental allele is called genomic imprinting (Surani 1998). The *M6p/Igf2r* gene is imprinted in mice (Barlow et al. 1991) and is expressed only from the maternal allele in all tissues except potentially the brain (Hu et al. 1998). In contrast, imprinting of the M6P/IGF2R gene is a polymorphic trait in humans, with most people having biallelic expression in all tissues (Xu et al. 1993). Although imprinting of the M6P/IGF2R may have provided an evolutionary advantage to the mother during the emergence of mammalian intrauterine growth (Haig and Graham 1991), monoallelic expression of this gene would also be predicted to increase cancer susceptibility. In support of this postulate, Xu et al. and associates (1997) have recently demonstrated M6P/IGF2R imprinting in 50% of Wilm's tumors.

Plomin and his colleagues have also identified the M6P/IGF2R as a putative "IQ gene" (Chorney et al. 1998). By comparing Caucasian children with an IQ of 160 or higher with those with an average IQ, they showed that the M6P/IGF2R is linked to human cognitive ability. If a polymorphism in the M6P/IGF2R coding sequence or its regulatory region is subsequently shown to functionally affect IQ, it would be the first identified gene known to contribute to intelligence. Thus, the M6P/IGF2R plays a fundamental role in biological processes ranging from embryogenesis to carcinogenesis. How the M6P/IGF2R could possess such diverse biological functions is an intriguing enigma that remains to be solved.

To facilitate future studies on the human M6P/IGF2R, we have characterized its complete genomic structure. The human cDNA sequence was divided into 48 exons based upon the mouse genomic structure (Szebenyi and Rotwein 1994), and PCR primers were designed to traverse these putative introns. All the introns except 1, 2, and 4 were amplified from human genomic DNA with either long-template PCR (Boehringer Mannheim Corp., Indianapolis, Ind.) or traditional PCR (Qiagen, Inc, Santa Clarita, Calif.). PCR products were sequenced on an ABI automated sequencer (Perkin-Elmer Corp., Foster City, Calif.). The sizes of introns 1 and 2 and their 5′ and 3′ boundary sequences were previously defined (Riesewijk et al. 1996; Smrzka et al. 1995); this information is available on GenBank (Accession Nos. X83699, X83700, and X83701). To sequence intron 4, human BAC clones (174E20 and 650K6) were first obtained by probing filters from Research Genetics, Inc. (Huntsville, Ala.) with an RT-PCR product spanning exons 1 to 6. A 13-kb stretch of DNA containing intron 4 was then PCR amplified from this BAC clone and the exon-intron boundaries sequenced. With the exception of intron 15, all exon-intron splice sites (93/94) conformed to the AG/GT rule (Mount 1982). Interestingly, the single intron splice site exception is also present in the mouse *M6p/Igf2r* homolog (Szebenyi and Rotwein 1994).

Intron sizes were determined by sequencing the entire intron or estimated by electrophoresis. Twenty introns were sequenced entirely (that is, introns 8, 9, 13–17, 21, 23, 24, 27, 28, 32–34, 37, 38, 42, 43, and 46), and at least 100 bp of flanking intronic sequence were determined for the remaining introns. The entire human M6P/IGF2R gene was then reconstructed with the use of the previously

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Table 1. Human M6P/IGF2R intron-exon boundaries.

Exon	cDNA (nt)	Exon size (bp)	5′ splice donor	Intron size (kb)	3′ splice acceptor	Intron phase[a]
1	1	296	CAGgtgggtggcccgccc	22.0	tttcttcctttccagTTA	2
2	296	140	TGGgtaagtagaactacc	17.0	tttctctccaaatagGTG	1
3	437	125	CTGgtgagtccacacagg	1.5	attttttttaatagGGA	0
4	562	99	GAGgtaacatgggaactt	13.0	cttcctccctccagGTG	0
5	661	133	TAGgtatgaatctttgtg	2.0	atacatgattttcagACA	1
6	794	130	CAGgtcagtcaaggcctc	2.2	atgtggctctcccagGCT	2
7	924	106	GAGgtaagtgactcgtct	2.7	cattgttcctgatagGGC	0
8	1030	163	GAGgtaagcaggtgcttt	0.227	tttccctgtttttagGTT	1
9	1193	166	CCGgtacgtcaacaacct	1.315	ttcacaaaaatctagATA	2
10	1359	104	CAGgtaagtgtgcgctgg	5.5	tcttgaattgtgcagGTA	1
11	1463	165	CAGgtactgccctccttg	2.5	tttgttttgttacagAAC	1
12	1628	141	TGGgtgagttgtgcctgg	1.2	ccctttctcttccagATA	1
13	1769	144	CAGgtaaaaattttaaaa	1.095	tttccccattgacagGTG	1
14	1913	138	CAGgtctgtgtccaagca	0.614	tttaatctttctaagGGT	1
15	2051	148	AAGgcaagtagcttctca	0.511	cgtgtgttaattcagTGA	2
16	2199	178	CAGgtaggaatgtttgtc	0.456	ctgccgtgggattagGAA	0
17	2377	116	CAGgtgaggcagagtcag	0.468	gctttgaaattttagTCT	2
18	2493	169	CAGgtgaatctgttttca	2.0	ctggttttcttgcagGGC	0
19	2662	180	CTGgtaaggcactgctgc	5.0	gatttgcccattcagAAC	0
20	2842	102	CAGgtacgtgtgctttca	1.5	ttttgtttcctgtagGCT	0
21	2944	102	ATGgtaagagcgatatga	0.78	ccgtctgacctgcagTTT	0
22	3046	193	AAGgtgagctcagagcca	1.6	cttcttgctttacagGTA	1
23	3239	171	CCGgtaaggccgtgcggc	0.781	tttgttgtgtttcagACC	1
24	3410	144	AGGgtgagttctccttgg	0.105	attctgttcttccagGCA	1
25	3554	176	TCGgtgtgtgttcagacc	0.8	tgatttatattacagGGC	0
26	3730	88	AAGgtaggactgggcctg	1.5	tccatgttcttgaagGGG	1
27	3818	216	CAGgtaccattgtttgtc	0.771	tctccttcttacagGTC	1
28	4034	131	CGGgtgagcatgtaccga	0.271	tcttaacttttttagCCA	0
29	4165	98	CAAgtaagtccatggatg	3.0	ttttcttcttttcagAGA	2
30	4265	137	CTGgtgagagagggcctc	1.5	tgtctggtgctgcagAGC	1
31	4400	191	GTGgtaagggactgttcc	1.5	tgtgctttgttgtagAAC	0
32	4591	127	CAGgtgagaggtggtgcc	0.732	tcttccaccctacagGAC	1
33	4718	120	TGGgtgagtgctgtggtc	0.327	tcccttgtggtgcagGGG	1
34	4838	257	GCGgtgagttttcagatg	0.286	tttcctacttaacagACC	0
35	5095	219	ATAgtaagtatgacaaat	2.0	tcgctctttgtttagGAT	0
36	5344	150	ATGgtaagtgtgggcctg	2.3	tcttcctggcaacagGGA	0
37	5464	162	AAGgtaatgcgttcaccc	1.233	atggttttgtccagGTG	0
38	5626	208	CAGgtaaatatttgaaat	0.341	tcttcttctttccagAAA	1
39	5834	147	ACTgtgagtaggacggct	3.6	ccntacactccccagCAA	1
40	5981	235	CTCgtgagtgccttccca	1.0	tgtgtcgttttctagGTA	2
41	6216	137	TAGgtaaggcctgtgggt	3.0	atttgtgtgtttcagGTG	1
42	6353	115	GAGgtcaggagactgggg	0.957	cttccctcctcctagGTT	2
43	6468	147	TAAgtaagtaaaacgttt	0.662	tttctgtctcttcagGCT	2
44	6615	188	AAGgtaatccgtggcttc	6.5	cttcccgtatgacagACG	1
45	6803	187	GGGgtgagtatgaaatcc	5.5	gtgacgtccttgcagGGT	2
46	6990	153	GAGgtaagcgggtggcag	1.081	ccttttttttatagGGA	2
47	7143	70	AAGgtaattttctgtggc	1.8	gttgatccctggcagGTG	0
48	7213	1878				

[a] Intron-exon splice junctions are categorized as phase 0, uninterrupted codon; phase 1, codon interrupted after the first nucleotide; and phase2, codon interrupted after the second nucleotide.

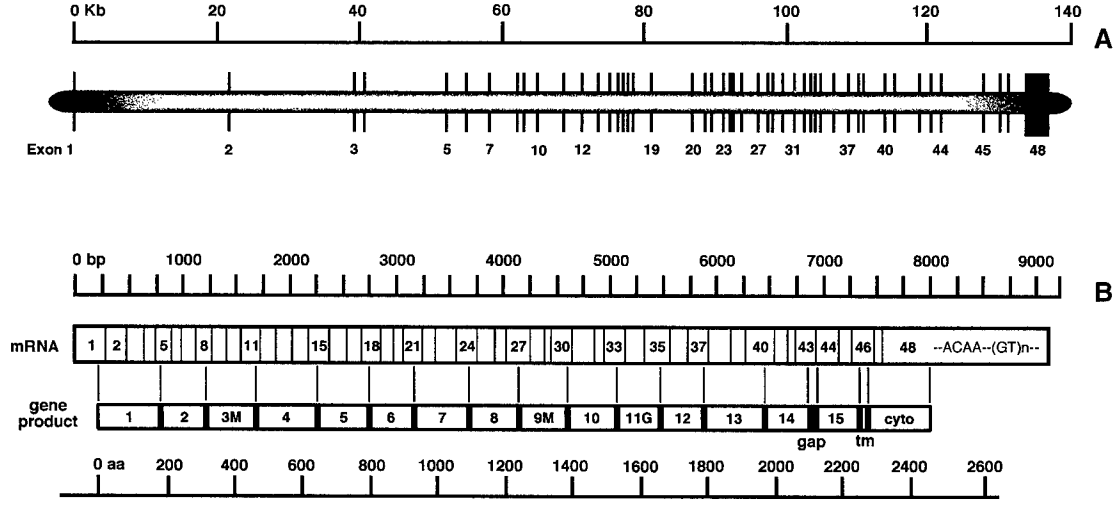

Fig. 1. Genomic organization of the human M6P/IGF2R. **A)** The 48 exons of the human M6P/IGF2R are distributed over approximately 136 kb. **B)** The mRNA is subdivided into exons, and the ACAA tetranucleotide insertion (Hol et al. 1992) and the $(GT)_n$ dinucleotide (Goto et al. 1992) polymorphic sites in the 3′-UTR (exon 48) are shown. The mRNA is also aligned with the protein sequence, which is divided into 15 structural repeats, each of approximately 150 amino acids; a 27-amino acid gap between repeats 14 and 15 (gap); a single 23-amino acid transmembrane domain (tm); and a 164-amino acid cytoplasmic tail (cyto) (Morgan et al. 1987). Repeats 3 and 9 (M) contain the M6P-binding domains, and repeat 11 (G) forms the IGF2-binding domain (Dahms et al. 1993; Schmidt et al. 1995).

Table 2. Intronic oligonucleotides for PCR amplification of the 48 human M6P/IGF2R exons.

Exon	F1 (5′ to 3′)	R1 (5′ to 3′)	Nested Primer (5′ to 3′)[a]
1	gctgtcacgtgacgcggttc	accgcgagcggagcctgcgtc	gccgccgctgccgctgtcgcF2
2	ctagtttgtggcagttttaagcaaatg	attgccaatccagtaatttcagg	gataaatcagtgacattgacaagttgF2
3	ggttatgtatgttttatagcctg	ttaaagaaatacatcaagtgcctgtg	aatacatcaagtgcctgtgtggacR2
4	tttattttagtagcctttactgcattctca	gataaagttcccagagtatacttc	ccagagtatacttcataagcatgR2
5	ctgattgaccaagatgtatactg	gaaaggcaaagatattgaagccatag	cctcaccaccacccccctcagR2
6	ctaagggtacgtgtgattatcactc	gaaagtcaggtccttgctggag	aaacgccaacagcatcggaggR2
7	ggcaacatatgaatttggatgtac	caacccaggctactgtgcgc	gtgcgccacattagtgatcaggR2
8	gtggaaaatctgcattaagctgcatg	ccttcttcctaagcagcgcc	caggaggcagaaagcacctgcR2
9	gactaagtaagactgtaatcttctaatacc	cgcacagaggttgttgacgtac	aatacctattcatataaaacaagcctcF2
10	cccaaacacatttgtctgtgtattc	acaagcacatgcccatgaatgc	gcaaagaggaggggctgaacR2
11	gctgttcctcaattttggtcacg	gaaaatgttccatgagcatgtggac	gactaagacccgcatggcaaggR2
12	gtgactcagagaaatcagcattgc	ctaactcattccaaactggatgcc	gaaaagcatcacctagatcttccR2
13	gtcacttctttgtctgcgtgatgatc	atatgaagaatgcaggacctctgg	caggacctctggcagaagccR2
14	gtcccttccaagtctacttctagc	gtggttccaagtcacattaaagcag	cacattaaagcagaggtcctgcR2
15	gttgggaacctcctgggaag	cttagcatataatgcctaagaatgaaac	ctaagaatgaaacagaactgagaagcR2
16	gtgactcctcacgtcgctcacg	cacaggcatgagtatcctcagg	gtatcctcagggagcgcgatgR2
17	ctcattgggaacattgctctcg	cagcaacacttgcaacctcagc	caacctcagcatctgtaccagR2
18	gtaagctttacttccccaactacatag	ctcccactaagtcatggaattagg	taggagggcaaaggagacaagcR2
19	ccaccaataacgaatcgactg	cacataaggcaacgctcagtc	aatgcagcagtgaaggtcaccR2
20	agtattcttttggttctatcaagttcc	gtccagcaggcagctcagc	ctcagcacgagggccaggtgR2
21	gtgctgtatgtatgttatgttcctgtg	gtgacttcaataatgcactctcc	ctgtttcaaagcaaactggaaatgcR2
22	tcttgtctgtggtgagatacgagg	ctacactcaggaaggtgcatggc	atggccagccacagagccacR2
23	ctgcactgtgcttgtgggctgc	gactcttgaccggcctctcagttc	gaccggcctctcagttcttaggR2
24	gcagttcttgagtgctcacaagg	caaatgctctaataaatgaacagacgc	cagacgccaatcaagagaccaagR2
25	gagcatttgactcaaggtcatcgc	gaaatgggaaatggagtcacccg	gggacaacatctcattgctggtcR2
26	gattacaggtgtgagccaccgtg	catcgttcagaacgtctgctactctatac	aaaatgacttgtagggacaggcR2
27	cgtgtgtggttgcagttgcc	ctcaaggaaaattcatcttcagc	gttgcagttgcccttcacttcF2
28	gtgtcacatgtcttaggctaag	aatatgatcccagcagcctgag	gtttgacagcctagggacF2
29	caaagtataaactaaagttttgcattctctac	gcaaaatcatatccaagttagtctgtcc	gttttgcattctcactttatatatgtgF2
30	acgaccaagcctaactaactgc	ctctcaccagtgctggcctgtg	cctgtgatacactcactgcaaacR2
31	tgatgaagttctgttctagcctg	gcagaaggtgcaggaacagtccc	gggagtcactaaaggcaactcF2
32	ccacttgtaagttgaaatcatgatagac	caccgtctctcacctgcgctg	gctggggttaacgactggcacR2
33	gcctcccaagtctcagctcc	gcaaagagactgaggacaacc	ccctggagtcactgtgtccF2
34	gaaattgatggtcctgacttgcg	gcactggagatgcacttctcc	catcagaaaattggccatcgagtcF2
35	ggatgacctagtggtgattagg	cgcaaaggttatcactaaaagtcttgc	gtcttgcaaaaattatctccacccttgR2
36	ccttgggaatggttaatttcctg	cgcactttctggttgtctagcc	gccttggctcactaacccgcagR2
37	tgtcactgctatctatccctatgcc	cagcacctgcgcacctggtacacc	gtacacctgcaccagcaacgR2
38	actttgagacctgggtgc	cagcttctttctgctgctatgtc	gctgccactctgctgacggcF2
39	ctgaggtgattgtgcctggcg	ctcagtgtgaatcgagccttgac	cgaggcacagctgccacactgF2
40	gcatagacacagtgacagtctgatc	gcagtctgaagttcacatgc	gttcacatgctgagggtgtggcR2
41	cagggcagagacgtcacttgc	cacctttcccggttcagatgctg	gttcagatgctgctccttgaaccR2
42	gaattgacaggtgtgagccactg	ctccagtatgctcactgcacag	gcacagtcccgctctgagccR2
43	gttttgcagtcttcccttatgtctg	gcattgctggtttaattttgaacac	catttcacagctcagaaggaaaacgR2
44	ctgagggtttatgtcatgaatgcc	ccttccttggaggaagttaaatgtg	gaagttaaatgtggaactttgtgggR2
45	ggagctaagctcagtctgctcgtg	cacttattcttaagggaagatgtgg	gatgtgggtacaagtgctaagcR2
46	caggttgtggctgtggcagc	tagctatggaggcatgcatcc	catccaccgccccacctcR2
47	ccatgccctctctacactggag	cctgatgagaacgacatggacagcg	ggcaggccttcaagagactcgR2
48	ggctcacgtggtctctgctgttg	gcctgacctcctcaccctc	gcatcatcgagtggaagtcR2

[a] Nested primers F2 and R2 should be paired with R1 and F1, respectively, during second-round PCR.

characterized genomic sequence for exons 1 to 3 (Riesewijk et al. 1996; Smrzka et al. 1995) and the sequence from our overlapping PCR-generated products. The complete set of intron-exon boundary sequences for the M6P/IGF2R and all of the intron and exon sizes are presented in Table 1. The codon position that is interrupted by the intron is indicated by the intron phase. The intron-exon splice junctions for the human M6P/IGF2R are identical in position to those in the mouse (Szebenyi and Rotwein 1994). The total size of the human M6P/IGF2R gene is estimated to be 136 kb (Table 1, Fig. 1). This is approximately 43 kb larger than that for the mouse gene, and introns 2 and 4 account for much of this increased size. The M6P and IGF2 receptor binding domains are also assigned to the appropriate exons (Fig. 1). The intron-exon boundary sequences were deposited with GenBank (Accession Nos. AFO69333-378), and will also be available on the M6P/IGF2R Information Core (http://www.radonc.duke.ed/~jirtle/homepage.html).

We have also designed intronic oligonucleotides for PCR amplification of the 48 human exons, including nested primers to allow for second-round DNA amplification (Table 2). DNA amplification from formalin-fixed, paraffin-embedded tissues requires primers that minimize the size of the amplified regions while including the splice sites flanking the exons. All 48 primer sets have been successfully employed to PCR amplify DNA isolated from either frozen tissue or formalin-fixed, paraffin-embedded samples. These nested primer sets yield a single PCR product approximately the size of the exon (Table 1) plus 80 bp. The PCR conditions used for all primer sets were 94°C × 20 s, 55°C × 30 s, and 72°C × 20 s for the 25-cycle first round and 30-cycle second round DNA amplifications.

In conclusion, we have determined the genomic structure and the intron-exon boundaries of the human M6P/IGF2R. These sequence data have been used to design PCR primers that allow for the systematic analysis of the 48 exons that encode for the human M6P/IGF2R gene. This should greatly facilitate linkage, phylogenetic, functional, and mutational analyses of the M6P/IGF2R, thereby enhancing further our understanding of this receptor's function in evolution, cancer biology, and human intelligence.

Acknowledgments. We thank Angus De Souza, Greg Falls, Douglas Marchuk, David Pulford, and Tomoya Yamada for their technical assistance; James V. Jirtle for M6P/IGF2R webpage design; Helena Abushamaa of the Duke Genetics Facility for help with BAC clone screening; and Marnee Baker and Scott Langdon for help with DNA sequencing. This study was supported by National Institutes of Health grants CA25951 and FS08823, Department of Defense Grant DAMD17-98-1-8305, Sumitomo Chemical Company, Ltd., and Zeneca Pharmaceuticals, Ltd.

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