Gene amplifications at chromosome 7 of the human gastric cancer genome

SANGHWA YANG

Cancer Metastasis Research Center, Yonsei University College of Medicine,
134 Shinchon-Dong, Seoul 120-752, Korea

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Abstract. Genetic aberrations at chromosome 7 are known to be related with diverse human diseases, including cancer and autism. In a number of cancer research areas involving gastric cancer, several comparative genomic hybridization studies employing metaphase chromosome or BAC clone microarrays have repeatedly identified human chromosome 7 as containing 'regions of changes' related with cancer progression. cDNA microarray-based comparative genomic hybridization can be used to directly identify individual target genes undergoing copy number variations. Copy number change analysis for 17,000 genes on a microarray format was performed with tumor and normal gastric tissues from 30 patients. A group of 90 genes undergoing copy number increases (gene amplification) at the p11~p22 or q21~q36 region of chromosome 7 is reported. The list of genes includes *wingless-type MMTV integration site family member 2* (WNT2), a proto-oncogene and *acyloxyacyl hydrolase* (AOAH) that was amplified in >80% of the tested cases. The amplified genes are those functioning in the biological processes such as signal transduction pathways, cell proliferation, metabolism, transport, inflammatory response and protein folding or proteolysis. Also found in the list are genes that are targets for drug development, such as *maltase-glucoamylase* (MGAM), *cyclin-dependent kinase 5* (CDK5), *neuropeptide Y* (NPY) and *dopa decarboxylase* (DDC). The current dataset can be used as one of the resources in understanding genetic aberrations of chromosome 7 in human gastric cancer.

Correspondence to: Dr Sanghwa Yang, Cancer Metastasis Research Center, Yonsei University College of Medicine, 134 Shinchon-Dong, Seoul 120-752, Korea
E-mail: ysh@yumc.yonsei.ac.kr

Abbreviations: aCGH, array comparative genomic hybridization; GB acc no, Genbank accession number; PCR, polymerase chain reaction; Mb, mega base pairs; Est, expressed sequence tag

Key words: gastric cancer, array comparative genomic hybridization, chromosome 7

Introduction

The completion of human genome sequencing and the subsequent gene annotations, together with a rapid development of high throughput screening technologies, such as DNA microarrays, have made it possible to perform genome-scale expression profiling and comparative genomic hybridizations (CGHs) in various cancer models. The elucidation of gene copy number variations in several cancer genomes is generating very informative results. Metaphase chromosome CGH and the recent introduction of BAC and especially cDNA microarray-based CGHs (aCGH) (1) have greatly contributed to the identification of chromosome aberrations and of amplified and deleted genes in gastric cancer tissues and cell lines (2-9).

Chromosome 7 has often been reported as containing regions undergoing genetic changes or unstable regions in various cancer models including gastric cancer (3,6,10-17). However, there have been no systematic searches for individual genes undergoing copy number increases or amplifications, since most of the previously mentioned studies employed metaphase chromosome or BAC array CGHs. Human chromosome 7 is ~159 Mb in length, contains 1150 genes and 940 'pseudogenes', a number of which have been implicated in a diversity of human diseases, including cystic fibrosis, deafness, B-cell lymphoma and cancers (18). Chromosome 7 contains known oncogenes that exhibit gene amplifications, including *epidermal growth factor receptor* (EGFR, located at 7p12), *hepatocyte growth factor* (HGF, 7q21.1) and *met proto-oncogene* (MET/HGFR, 7q31) (19-23). Since one of the most frequent mechanisms of oncogenic activation is gene amplification, it is of utmost importance to be able to identify all the possible collection of genes that are amplified in tumor tissues of a given cancer model. We previously reported a cDNA microarray-based CGH analysis of gastric cancer, in which gene copy number increases at chromosome 20 were identified (9) and the relationship between copy number changes and gene expression was explored (24). In the current analysis, we focused on the gene amplification at chromosome 7 of the gastric cancer genome, which could potentially lead to the identification of novel candidate oncogenes.

Materials and methods

Gastric cancer tissues and aCGH. Gastric cancer microarray CGH experiments were described previously (9). Briefly, 30

pairs of normal gastric and corresponding tumor tissues were used for the experiment with patient consent and approval of the supervisory committee of the Yonsei University College of Medicine. The tumor tissues used for the experiments contained at least 70% tumor content as was confirmed by a pathologist. Tissues were kept at -80˚C after surgery, ground to powder using liquid nitrogen, pestle and mortar, and the genomic DNA was prepared using the phenol/chloroform/isoamylalcohol method. CGH experiments were performed using 17K cDNA microarrays (GenomicTree, Daejeon, Korea), which have been employed in several experiments and have produced reliable data sets (25-27). The microarrays contain PCR products from 17,000 Ultimate™ ORF clones, which are full-insert sequenced and were confirmed for a 100% match to known amino acid sequences by the manufacturer (Invitrogen, Carlsbad, CA). The OmniGrid™ microarrayer (GeneMachines Inc., San Carlos, CA) and CMT-GAPS™ glass slides were used for printing the microarrays (Corning, Charlotte, NC). Genomic DNA (6 μg) was *Dpn*II-digested, purified using a QIAquick PCR purification kit (Qiagen, Düsseldorf, Germany) and was subjected to fluorescent labeling using a Bioprime labeling kit (Invitrogen). The reaction mixtures were incubated at 37˚C for 2 h in the dark and were quenched by adding 5 μl of 0.5 M EDTA, pH 8.0. The microarray slides were incubated in 3.5X SSC, 0.1% SDS with 10 mg/ml BSA for 1 h at 42˚C for blocking. The genomic DNA from the normal and tumor tissues was labeled with Cy3-dCTP and Cy5-dCTP, respectively. Cy3- and Cy5-labeled DNAs from the tissue samples were mixed with 30 μg human Cot-1 DNA, 20 μg poly(dA)-poly(dT) oligonucleotides and 100 μg yeast tRNA. A Microcon-30 filter (Amicon, Bedford, MA) was used to purify and concentrate the hybridization mixture, which was then adjusted to the final concentration of 3.5X SSC and 0.3% SDS in a volume of 60 μl. Following denaturation at 100˚C for 1.5 min and pre-annealing at 37˚C for 30 min, the labeled DNA mixture was applied to the cDNA microarray and incubated at 65˚C for 20-30 h. The slides were then washed for 2 min each in 0.5X SSC/0.01% SDS, 0.06X SSC/0.01% SDS, and 0.05X SSC, consecutively, at room temperature and spun-dried. The microarray slides were scanned using a GenePix 4000B scanner (Axon Instruments, Foster City, CA), and the data were saved in a Gene Pix Result (GPR) format. The microarray dataset was deposited in the ArrayExpress database (www.ebi.ac.uk).

Data analysis. Copy number change analysis for the genes located on chromosome 20 and the genome-scale correlation analysis of copy number change and gene expression from the same data set were recently published (9,24). In this report, gene copy number increases for genes located on chromosome 7 are shown in detail. Previously, we reported that 1,780 genes (probes with unique GB acc nos) showed copy number increases in gastric cancer, when 30 normal and 30 tumor tissues were analyzed by aCGH on 17K cDNA microarrays. From 1,780 probes with unique GB acc nos, copy change ratios for the 104 probes on chromosome 7 were extracted. After Source annotation (http://genome-www5.stanford.edu/cgi-bin/source/sourceSearch), 90 genes with reliable information including gene symbols, Unigene IDs and cytoband information were selected. For the visualization of the copy change data in CGH-Explorer (28), which requires nucleotide position information to plot the relative distance of the genes in the analysis, the absolute nucleotide positions for the start point of each gene were obtained from Ensembl (www.ensembl.org). The gene copy number changes detected by the current aCGH were validated by real-time PCR with tissue genomic and also by fluorescent *in situ* hybridization (24). Copy number increases were defined as those showing $\log_2$(Cy5/Cy3) ≥0.58 (~1.5-fold).

Results and discussion

Global view of gene amplifications on chromosome 7 of the gastric cancer genome. When 30 pairs of normal and tumor tissues were tested for copy number variations in gastric cancer using 17K cDNA array in a direct design (normal-Cy3 and tumor-Cy5), it was shown that 1,780 genes were undergoing copy number increases (gene amplifications) in gastric cancer. Of these, 90 chromosome 7-specific genes were used for further analysis (Materials and methods). First, to look at the empirical distribution of the copy change ratios for the 90 genes, a density histogram of normal distribution was drawn (Fig. 1A). Since we were testing only the amplified genes, and not the deleted genes, the histogram showed skewness to the positive log ratio values in reference to 0 ('skew': 1.598) and also showed a positive kurtosis, in that there was a heavier tail than for a normal distribution ('kurt': 7.515). The mean $\log_2$(Cy5/Cy3) was 0.254 with a variance of 0.283 for the dataset, and it matched our definition of copy number increase or gene amplification; in control CGH experiments employing two normal gastric tissues from male and female subjects (XY and XX), variations in $\log_2$(Cy5/Cy3) ratios between -0.3 and +0.3 were observed for the genes located on autosomes (26), and adding one standard deviation we defined copy number increase in this data set as those with $\log_2$(Cy5/Cy3) ≥0.58 (9).

To visualize the overall distribution of genes and their ratios, additional graphs were generated. The 90 genes were given their absolute position from pter to qter of chromosome 7 in nucleotide numbers and were drawn in their relative positions on chromosome 7. A gene density map (Fig. 1B) shows a distribution of genes being tested on a chromosome. In the case of chromosome 7, 90 genes were evenly distributed along chromosome 7. When the number of the amplified genes was plotted on the cytoband location of chromosome 7, it was shown that amplified genes were centered between p11~p22 and q21~q36 (Fig. 1C). A moving average plot (Fig. 1D) shows 30 copy number change profiles representing 30 experiments (patients) over 90 genes, also along the chromosomal locations. Some genes showing a high degree of amplification, or high log ratios, are shown, including *anterior gradient homolog 2* (AGR2), *acyloxyacyl hydrolase* (AOAH), *LanC lantibiotic synthetase component C-like 2 (bacterial)* (LANCL2) and *split hand/foot malformation (ectrodactyly) type 1* (SHFM1). Although some public databases indicated that LANCL2 is located at 7q31.1-q31.33, the Ensembl database with the latest update on human genome sequencing information showed that the starting and stop positions of the LANCL2 genomic clone are

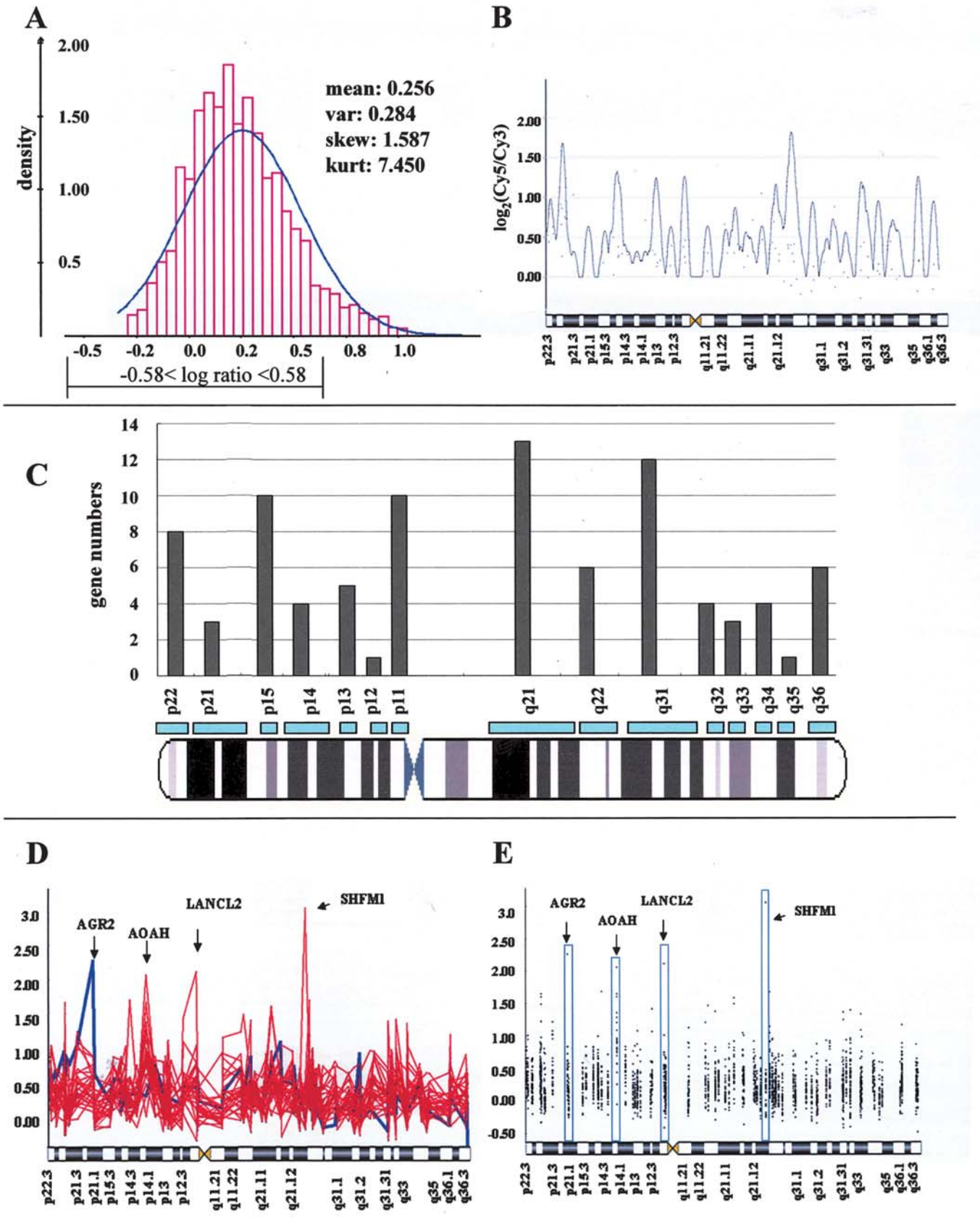

Figure 1. Gene copy number increases in chromosome 7 as visualized by CGH-Explorer. (A) Distribution of ratio data for 90 genes amplified in chromosome 7. A density (of log ratios, on X-axis) histogram is shown, together with a Gaussian density (Y-axis) fitted to the data. The mean, variance (var), skewness (skew) and excess kurtosis (kurt) of the data are shown in the upper right corner. Log ratios between -0.58 and +0.58 were considered as noise signal. A bar-line is shown at the bottom of the histogram to indicate the range. (B) Gene density histogram. The spatial distribution of 90 genes, located at relative nucleotide positions on chromosome 7 is shown with Epanechnikov kernel density estimator. (C) Gene amplification and chromosome cytogenetic location. Gene cytogenetic location information was simplified (7q11.23→7q11) and combined gene numbers are shown along the cytoband. (D) A moving average plot. A line plot of the 90 (gene) ratios is shown for all of the 30 arrays (patients). One of the 30 array plots is shown in blue color for ease of visualization. Four of the genes with log ratios >2 at least once are shown. (E) A scatter plot of the copy change ratios. Thirty ratios are shown as dots for each gene.

Table I. Amplified genes in chromosome 7 of the human gastric cancer genome.

GB Acc no	Name	Symbol	Location	Case	Ratio[a]
AI479904	Split hand/foot malformation type 1	SHFM1	7q21.3	1	8.34
AA699441	Caspase recruitment domain family, member 4	CARD4	7p15	1	3.23
AA459308	Elastin	ELN	7q11.23	1	2.77
AA076063	Caldesmon 1	CALD1	7q33	2	2.32
AA626787	Ras-related C3 botulinum toxin substrate 1	RAC1	7p22	10	2.13
AA629584	ADP-ribosylation factor 5	ARF5	7q31.3	3	2.12
AA670434	Brain protein I3	BRI3	7q21.3	6	2.11
T65864	Acyloxyacyl hydrolase	AOAH	7p14	25	2.02
AI361688	LanC lantibiotic synthetase component C-like 2	LANCL2	7q31.1	6	2.02
AI088691	Solute carrier family 25, member 40	SLC25A40	7q21.12	1	2.01
AI671991	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A	SEMA3A	7p12.1	7	2.00
AW073291	Anterior gradient homolog 2	AGR2	7p21.3	6	1.98
AI681896	Glucuronidase, ß	GUSB	7q21.11	1	1.87
AI335654	PHD finger protein 14	PHF14	7p21.3	8	1.86
AA878093	Scavenger receptor cysteine-rich domain containing, group B (4 domains)	SRCRB4D	7q11.23	4	1.86
AW072169	Zinc finger, MIZ-type containing 2	ZMIZ2	7p13	2	1.85
AA461476	DEAD (Asp-Glu-Ala-Asp) box polypeptide 56	DDX56	7p13	1	1.84
AA126919	Tyrosylprotein sulfotransferase 1	TPST1	7q11.21	4	1.84
AA626867	KDEL (Lys-Asp-Glu-Leu) ER protein retention receptor 2	KDELR2	7p22.1	1	1.82
AA906730	Ca^{2+}-dependent activator protein for secretion 2	CADPS2	7q31.3	5	1.81
AI261686	Dopa decarboxylase	DDC	7p11	5	1.81
AA192419	Biliverdin reductase A	BLVRA	7p14	1	1.80
AI370177	Transmembrane protein 140	TMEM140	7q33	3	1.75
AA774034	Coatomer protein complex, subunit γ 2	COPG2	7q32	14	1.75
AA864226	Suppression of tumorigenicity 7	ST7	7q31.1	2	1.75
AA887211	ATP-binding cassette, sub-family B (MDR/TAP) 1	ABCB1	7q21.1	5	1.75
AA810225	G protein-coupled receptor 30	GPR30	7p22.3	4	1.74
AI199184	Actin-related protein 2/3 complex, subunit 1A	ARPC1A	7q22.1	3	1.73
AA864398	BAI1-associated protein 2-like 1	BAIAP2L1	7q21.3	1	1.73
AA428361	Amiloride-sensitive cation channel 3	ACCN3	7q35	9	1.72
AA055491	Tetraspanin 12	TSPAN12	7q31.31	2	1.71
AI244357	GRIP and coiled-coil domain containing 1	GCC1	7q32.1	1	1.71
AW004716	Proteasome subunit, α type, 2	PSMA2	7p13	1	1.71
AI583024	POM121 membrane glycoprotein	POM121	7q11.23	8	1.69
AI676154	Homeobox A9	HOXA9	7p15	2	1.68
AI360484	Calcium channel, voltage-dependent, α 2/δ subunit 1	CACNA2D1	7q21	4	1.68
AA406040	BUD31 homolog (yeast)	BUD31	7q22.1	3	1.68
AI675889	Neuropeptide Y	NPY	7p15.1	3	1.68
AI675658	Filamin C, γ	FLNC	7q32	5	1.68
R53455	Carboxypeptidase, vitellogenic-like	CPVL	7p15	2	1.68
AI630806	KIAA0644 gene product	KIAA0644	7p15.1	6	1.67
AA173290	Homeobox A1	HOXA1	7p15.3	7	1.66
AA975832	Glucocorticoid-induced transcript 1	GLCCI1	7p21.3	1	1.66
AI339538	Solute carrier family 26, member 3	SLC26A3	7q31	2	1.66
AI340575	Centaurin, γ 3	CENTG3	7q36.1	1	1.65
AI971229	FK506 binding protein 9, 63 kDa	FKBP9	7p11.1	1	1.65
AA489199	Patatin-like phospholipase domain containing 8	PNPLA8	7q31	1	1.65
AI650647	A kinase (PRKA) anchor protein (yotiao) 9	AKAP9	7q21	7	1.64
H59259	Replication protein A3, 14 kDa	RPA3	7p22	3	1.64
AI356429	JTV1 gene	JTV1	7p22	5	1.64

Table I. Continued.

GB Acc no	Name	Symbol	Location	Case	Ratio[a]
AA917769	Cell division cycle 2-like 5	CDC2L5	7p13	6	1.64
AI339140	Protein kinase, cAMP-dependent, regulatory, type I, ß	PRKAR1B	7p22	6	1.64
AA872041	Ubiquitin-specific peptidase 42	USP42	7p22.1	7	1.63
AA401479	Cyclin-dependent kinase 5	CDK5	7q36	1	1.62
AA233185	Insulin-like growth factor binding protein 1	IGFBP1	7p13	2	1.62
N29844	Peptidase (mitochondrial processing) ß	PMPCB	7q22.1	1	1.62
AA446477	DnaJ (Hsp40) homolog, subfamily B, member 9	DNAJB9	7q31	1	1.61
AI433283	GATA zinc finger domain containing 1	GATAD1	7q21	1	1.61
AA894865	Chromosome 7 open reading frame 13	C7orf13	7q36	3	1.61
AI668639	MyoD family inhibitor domain containing	MDFIC	7q31.1	1	1.60
N75028	Phosphoserine phosphatase-like	PSPHL	7q11.2	1	1.60
AA962213	Cut-like 1, CCAAT displacement protein	CUTL1	7q22.1	3	1.59
AI299566	Paternally expressed 10	PEG10	7q21	1	1.59
N68166	G protein ß polypeptide 2	GNB2	7q21.3	1	1.58
AA488432	Phosphoserine phosphatase	PSPH	7p15.2	1	1.58
AI301329	Aldo-keto reductase family 1, member B10	AKR1B10	7q33	1	1.57
AI143715	Caspase recruitment domain family, member 11	CARD11	7p22	3	1.57
AA872690	Chaperonin containing TCP1, subunit 6A	CCT6A	7p11.2	1	1.57
AA521339	Chimerin 2	CHN2	7p15.3	1	1.57
AA983626	Nucleolar protein with MIF4G domain 1	NOM1	7q36.3	1	1.57
AA432066	Sarcoglycan, ε	SGCE	7q21	1	1.57
AA992441	COP9 constitutive photomorphogenic homolog subunit 6 (Arabidopsis)	COPS6	7q22.1	1	1.56
AI300449	Guanine nucleotide binding protein, α transducing 3	GNAT3	7q21.11	1	1.56
AI215853	Zinc finger protein 800	ZNF800	7q31.33	2	1.55
AA865558	C-type lectin domain family 2, member L	CLEC2L	7q34	4	1.54
AA485431	G protein α 12	GNA12	7p22.2	3	1.54
AI214268	Poly(ADP-ribose) polymerase family, member 12	PARP12	7q34	1	1.54
N78828	Wingless-type MMTV integration site family member 2	WNT2	7q31	1	1.54
AA598610	Mesoderm-specific transcript homolog	MEST	7q32	1	1.53
AA400474	Zona pellucida binding protein	ZPBP	7p14.3	1	1.53
AI310513	Transmembrane protein 60	TMEM60	7q11.23	2	1.52
AA894763	Maltase-glucoamylase	MGAM	7q34	1	1.52
AA482079	Replication initiator 1	REPIN1	7q36.1	1	1.52
AA863470	Family with sequence similarity 126, member A	FAM126A	7p15.3	1	1.51
AI972286	Prolactin-induced protein	PIP	7q34	1	1.51
AW072305	Zona pellucida glycoprotein 3	ZP3	7q11.23	1	1.51
AI522064	Hypothetical protein FLJ31818	FLJ31818	7q31.1	3	1.50
AA025940	Aryl hydrocarbon receptor	AHR	7p15	1	1.49
AA431203	DnaJ homolog, subfamily B, member 6	DNAJB6	7q36.3	1	1.49
AI620493	Erythropoietin	EPO	7q22	1	1.49

[a]Ratio refers to fold increases. Genes with $\log_2$(Cy5-tumor/Cy3-normal) ≥0.58 are listed in the order of fold change.

55,400,635 and 55,468,929 nucleotides, respectively, from the pter of the chromosome, which corresponds to a cytoband location of 7p11.2. There were differences even for the 4 amplified genes shown as an example in Fig. 1C. While all 4 genes of AGR2, AOAH, LANCL2 and SHFM1 showed a high level of gene copy number increase, $\log_2$(Cy5/Cy3) ≥2, indicating >4-fold amplification, only the AOAH gene was being amplified with a high frequency. In fact AOAH represented the most frequently copy number increased gene on chromosome 7, being amplified in 25 of the 30 tested patients (~83% frequency), while SHFM1 showed the highest increase in ratio (8-fold increase in tumor tissue) in

Table II. Amplified genes that are also therapeutic targets in other diseases.

Target name	Related disease	Drugs/ligands
Maltase-glucoamylase (MGAM)	Diabetes mellitus	Acarbose Miglitol Voglibose
Cyclin-dependent kinase 5 (CDK5)	Alzheimer's disease Bladder cancer	R)-roscovitine Alsterpaullone Deschloroflavopiridol Flavopiridol Hymenialdisine Indirubin-3'-noxime Indirubin-5-sulfonate Olomoucine Purvalanol B SU9516 Aminopurvalanol
Neuropeptide Y (NPY)	Heart failure Hypertension Obesity	
Dopa decarboxylase (DDC)	Parkinson's disease	Benserazide Carbidopa

Genes listed in Table I were searched for along with drugs/ligands in the Therapeutic Target Database maintained by Dr Chen Yuzong at the National University of Singapore (32), and 4 target genes were identified and listed. Neuropeptide Y is a target drug currently under investigation.

only one of the patients (Table I). When all 30 ratios for each of the 90 genes were plotted, it showed that AOAH had more amplified cases (Fig. 1D).

Amplified genes of chromosome 7 of the gastric cancer genome. In this study, we identified a total of 90 genes that were copy number increased in chromosome 7 with varying frequencies (Table I). The list contained such genes as *wingless-type MMTV integration site family member 2* (WNT2), a gene previously identified as an amplified proto-oncogene in gastric adenocarcinoma (11). We also recently identified genes that showed mRNA expression and copy number change profiles in a highly correlated manner (24). Of the 90 genes shown in Table I, five known genes and one Est were among the 158 genes that demonstrated correlation coefficients >0.6 between gene copy change and mRNA expression. For these genes, the copy number increase directly influenced the overall mRNA production from the corresponding gene. These include *guanine nucleotide binding protein* (*G protein*), *ß polypeptide 2* (symbol GNB2, GB acc no, N68166, correlation coefficient 0.72), *COP9 constitutive photomorphogenic homolog subunit 6* (COPS6, AA992441, 0.733), *chaperonin containing TCP1, subunit 6A* (ζ *1*) (CCT6A, AA872690, 0.767), *insulin-like growth factor binding protein 1* (IGFBP1, AA233185, 0.792), *replication protein A3*, 14 kDa (RPA3, H59259, 0.744) and an Est (AI630806, 0.765). The 90 amplified genes can be grouped into functional categories. Genes implicated in the signal transduction pathways include GNB2, RAC1, ARF5, CHN2, CENTG3, GPR30, WNT2, IGFBP1, AKAP9, GNA12, EPO and PRKAR1B. Other function groups representing members of the 90 amplified genes were cell proliferation (CDC2L5, CDK5, ELN, NPY, SEMA3A), cell metabolism (AKR1B10, DDC, GUSB, JTV1, MGAM, PNPLA8, PSPH), transport (BLVRA, COPG2, KDELR2, POM121, SLC25A40, SLC26A3), inflammatory response (AOAH, TPST1) and protein folding or proteolysis (DNAJB6, CCT6A, DNAJB9, FKBP9, PSMA2, CPVL, PMPCB, SHFM1, USP42).

Some of the selected genes are targets for therapeutic development in human cancers. For example, *ATP-binding cassette, sub-family B* (*MDR/TAP*), *member 1* (ABCB1), also called *multidrug resistance 1* (MDR1) or *P-glycoprotein 1*, encodes an efflux pump responsible for decreased drug accumulation in multidrug-resistant cells and confers multiple drug resistance in cancer chemotherapy (29). This gene is a target in the clinical study of multidrug resistance (MDR1) gene therapy, with promising initial results in breast cancer (30), but conflicting results in other cases (31). Other members of the 90 genes identified as being amplified in the gastric cancer genome in this report have also been targets of therapeutic development in other diseases (Table II). Genes on chromosome 7 have been implicated in various human diseases including cancer. The identification of chromosome 7-specific gene amplifications is useful data not only for gastric cancer research, but also for researching other human diseases.

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