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Deoxyribonucleic Acid Copy Number Aberrations in Vasospastic Angina Patients Using an Array Comparative Genomic Hybridization

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ABSTRACT

Background and Objectives: Vasospastic angina (VA) is a specific type of coronary artery disease and develops as a result of coronary artery spasm. Recently, a few studies have revealed that VA caused by coronary artery spasm is related to genetic traits. The objective of this study was to use the recently developed technique of array comparative genomic hybridization (CGH) to screen the genetic aberrations of VA. **Subjects and Methods:** To identify candidate genes that might be causally involved in the pathogenesis of VA, genomic deoxyribonucleic acids were extracted from whole blood of 28 patients with VA who presented at Department of Cardiology at Seoul St. Mary's Hospital, Seoul, Korea. The copy number profiles of these patients was then analyzed using array CGH and reverse transcriptase (RT) quantitative polymerase chain reaction (PCR). **Results:** Array CGH revealed gains in 31 different regions, with losses in the 4q35.2, 7q22.1, 10q26.3, 15q11.2, 16p13.11, 17p11.2 and 19q13.3 regions (more than 32% aberration in these regions). Several loci were found to be frequently including gains of 5p and 11q (50% of samples). The most common losses were found in 7q (54% of samples). Copy number aberrations in chromosomal regions were detected and corresponding genes were confirmed by RT quantitative PCR. The fold change levels were highest in the *CTDP1* (18q23), *HDAC10* (22q13.33), *KCNQ1* (11p15.5-p15.4), *NINJ2* (12p13.33), *NOTCH2* (1p12-p11.2), *PCSK6* (15q26.3), *SDHA* (5p15.33), and *MUC17* (7q22.1) genes. **Conclusion:** Many candidate chromosomal regions that might be related to the pathogenesis of VA were detected by array CGH and should be systematically investigated to establish the causative and specific genes for VA. **(Korean Circ J 2011;41:385-393)**

KEY WORDS: Array comparative genomic hybridization; Vasospastic angina.

Introduction

Vasospastic angina (VA), also called Prinzmetal's angina or variant angina, is a specific type of coronary artery disease and is developed due to not atherosclerosis but coronary artery spasm. This disease can cause acute coronary syndrome, including acute myocardial infarction, ventricular tachycardia, fibrillation or electro mechanical dissociation, and even sudden cardiac death.[1] The incidence of VA is higher in Asians than Caucasians or Blacks,[2][3] which strongly suggests that genetic traits might be involved in the pathogenesis of VA.[4-11]

Studies have shown that the loss of Kir6.1 {pore-forming inwardly rectifying K^+ (Kir) channel subunit} or SUR2 (sulfonylurea receptor regulatory subunit of K_{ATP} channel) by related gene deletions in mice induced VA.[4][5] So far, only these two genes have been shown to be associated with gene copy number alterations. Other investigations showed gene polymorphisms[6-8] and functional expression[9-11] in VA. Related gene polymorphisms in VA included an endothelial nitric oxide synthase gene,[6] adrenergic receptor gene,[7] and the paraoxonase 1 gene.[8] The Rho-associated kinase gene[9] and serotonin receptor ($5\text{-}HT_{1D\beta}$) gene[10][11] were also shown to be related to VA by their functional expression. To date, there have been no studies on deoxyribonucleic acid (DNA) copy number alterations in the whole human genome among patients

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• The authors have no financial conflicts of interest.

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with VA. The recent development of array-comparative genomic hybridization (CGH) provides a means to quantitatively measure DNA copy number aberrations and to map them directly to genomic sequences.[12-14] Also, tiling resolution DNA microarrays comprised of large-insert genomic clones such as bacterial artificial chromosomes (BACs) are a very useful and accurate method for detecting genomic aberrations in many genetic diseases.[15][16] Furthermore, array-CGH with BAC clones, has been proven to be a very useful and accurate tool for detecting genomic aberrations in whole blood samples for schizophrenia, epilepsy and mental retardation,[17-19] as well as in tissue from some cancers.[20-24] Both fluorescence *in situ* hybridization (FISH) and real-time polymerase chain reaction (RT-PCR) have also been applied empirically to confirm array-CGH results.[17][18][21-24]

In the study presented here, we analyzed genomic DNA copy number aberrations in whole blood from 28 patients with VA using array CGH. Genetic aberrations, for a subset of gained or lost gene, were confirmed by RT-PCR.

Subjects and Methods

Clinical description

Peripheral blood samples were collected from 28 patients with VA at the Department of Cardiology at Seoul St. Mary's Hospital, Seoul, Korea. In this study, the diagnosis of VA was made when patients met all of the following criteria: 1) repetitive burning or squeezing retrosternal chest pain, 2) definite positive response by coronary angiography with intra coronary acetylcholine provocation test, 3) no significant atherosclerotic stenosis in coronary artery (<50% narrowing of the coronary luminal diameter) according to quantitative coronary angiography. Blood samples were collected from 8 women and 20 men, aged 34-71 years (mean age±standard deviation, 56.44±11.4 in years). The clinical characteristics of the patients are shown in Table 1. Genomic DNA was extracted using a Puregene DNA isolation kit (Qiagen, Hilden, Germany). Reference DNA was pooled from 10 gender-matched (male), normal, healthy control subjects. All patients gave written informed consent to participate in this study. The protocol of this study was approved by the institutional review board of Seoul St. Mary's Hospital, The Catholic University of Korea.

Array comparative genomic hybridization

Array CGH analyses were conducted on 28 individual samples using commercial MACArray™ Karyo 4K BAC-chips (Macrogen, Seoul, Korea) with 4,030 BAC clones, in duplicate, on the whole human genome with a resolution of about 1 Mbp. Array CGH was performed as described previously.[25] Briefly, 500-700 ng target and reference DNAs were denatured in the presence of random primer and reaction buffer (BioPrime® DNA Labeling System, Invitrogen, Carlsbad, CA, USA) at 98-100°C for 5 minutes, and then cooled on ice for 5 minutes. The denatured DNA was differentially labeled with 3 μL of 1 mM Cy3- and Cy5-conjugated dCTP by random primed labeling (Perkin Elmer, Waltham, MA, USA). The mixture was incubated with a Klenow fragment at 37°C overnight. After labeling, unincorporated nucleotides were removed using MicroSpin™ G-50 columns (Amersham Biosciences, Buckinghamshire, UK). Cy3- and Cy5-labeled test DNA and reference DNA were mixed with 50 μL of human Cot-1 DNA for blocking of repeat sequences. After purification, this mixture was resolved in hybridization buffer (Macrogen) containing yeast tRNA for blocking of non-specific nucleotides binding.

Table 1. The clinical characteristics of patients with vasospastic angina

Characteristic	Value
Number of samples	28
Age, mean±standard deviation (years)	56.4±11.4
Male (%)	20 (71.4)
Current smoker (%)	11 (39.3)
Current alcohol drinking (%)	14 (50)

After the MACArray™-Karyo 4K BAC-chip was prehybridized in hybridization buffer with salmon sperm DNA for 1 hour, chips were hybridized with the purification mixture. It was then incubated for 72 hours in the 37°C hybridization chamber (BioMicro systems, Salt Lake City, UT, USA). After hybridization was complete, array chips were washed in 50% formamide-2x SSC at 46°C for 15 minutes, and 0.1% SDS-2x SSC at 46°C for 30 minutes. Next, the chips were washed in 50% sodium phosphate-0.1% NP40 for 15 minutes followed by washing with 2x SSC for 15 minutes at room temperature. After spin drying, hybridized arrays were scanned with a Genepix™ (Axon Instruments, Sunnyvale, CA, USA).

Data analysis

Raw signal intensities from the arrays were measured using the MAC Viewer v1.6.3 software (Macrogen). Log_2 (Cy3 intensity/Cy5 intensity ratios) were normalized by using the median of fluorescence ratios computed from the housekeeping DNA control fragments linearly distributed across the genome. Measurements flagged as unreliable by MAC Viewer v1.6.3 were removed from subsequent analysis. The threshold corresponds to 2 standard deviation (SD) values from the mean. The information on each individual clone was obtained from the UCSC Genome Bioinformatics database (May 2004 freeze, http://genome.ucsc.edu).

Reverse transcriptase quantitative polymerase chain reaction

To confirm the level of genomic imbalances identified by

array CGH in this study, DNA samples with obvious genomic changes were analyzed using RT quantitative PCR.[26] The reaction was performed in a total volume of 50 μL, including 25 μL of 2x IQ™ SYBR® green supermix (Bio-Rad, Hercules, CA, USA), 10 ng of DNA, and 10 pmol of each primer.[27] Forty cycles of amplification, data acquisition, and data analysis were performed in an iCycler (Bio-Rad). Primers for eight genes (*CTDP1, HDAC10, KCNQ1, NINJ2, NOTCH2, PCSK6, SDHA*, and *MUC17*) were selected and the position of each clone was obtained from the UCSC genome database (Table 2). The relative genomic copy number was calculated using the comparative CT Method.[28] GAPDH was used as an endogenous reference.[28-30]

Results

Genomic deoxyribonucleic acid copy number changes and pattern of aberrations

Chromosomal copy number changes were detected by array-CGH in VA. Copy number changes were presented as individual chromosome plots of $\log_2$ ratio of normalized Cy3 : Cy5 intensities versus chromosome regions for each of the individual 28 cases of VA. Table 3 shows the frequency of aberration, ranging from 7 (25% of chromosomal gain and loss) to 15 (54% of chromosomal loss at 7q) in 28 samples. Overall, the individual chromosomal aberration pattern was not random and tended to be consistent, showing DNA gains in 1p, 1q, 2p, 2q, 3p, 3q, 4p, 4q, 5p, 5q, 6p, 6q, 7q, 8p, 8q, 9q, 10p, 10q, 11p, 11q, 12q, 13q, 14q, 15q, 16p, 16q, 17p, 17q, 18q, 20p, 20q, 21q, and 22q; and showing DNA losses in 2p, 4q, 7q, 10q, 15q, 16p, 17p, and 19p. DNA gains, rather than DNA losses, were more frequently observed. The most frequent gain detected was at 5p15.33, and 11q12.2 (50%). Frequent DNA gains also were observed in 12p13.33, 14q32.33, and 15q11.2 in 13 samples (46%), and in 12p13.33 (43%), 1p12-p11.2, 4p15.1, 8q22.2, 11q25, and 15q26.3 in 11 samples (39%). Frequent gains (36%) were located at: 1q44, 3q24, 11q23.3, 15q11.2, 15q25.2, 15q26.3, 16p12.1, 16p13.2, 17p12, 18q12.3, and 21q11.2. Other regions with 25-32% frequency of DNA gains included 1p12-p11.2, 1p12, 1p21.1, 2p11.2, 2q21.2-q21.3, 2q22.1, 2q23.3, 2q31.1, 2q37.3, 3p14.1, 3q12.3, 4p15.1, 4p15.2, 4q24, 4q32.3, 4q34.3, 4q35.2, 5p12, 5p15.2, 5p15.33, 5q15, 6p12.2, 6q24.1, 7q11.23, 7q22.2, 8p23.3, 8q24.3, 9q22.1, 9q22.32, 9q33.3, 10p15.3, 10q11.23, 10q23.1, 10q26.3, 11p15.5, 11p15.5-p15.4, 11q14.1, 11q23.1, 11q23.2, 12p13.33, 12q22, 13q33.3, 14q31.3, 15q26.3, 16p11.2, 16q24.1, 17q24.3, 17q25.3, 18q23, 20p12.2, 20q12, 20q13.33, 21q11.2, 21q22.3, 22q12.2, and 22q13.33. The most common genomic losses were observed in 7q22.1 (54%), 4q35.2 (43%), and 15q11.2 (39%), 10q26.3 (36%). Other regions with 25-32% frequency of DNA losses included 2p24.1, 7q36.3, 10q26.3, 16p13.11, 16p13.3, 17p11.2, and 19p13.3 (Table 3). Fig. 1 shows chromosomal aberration regions. 1p12-p11.2, 5p15.33, 11p15.5-p15.4, 12p13.33, 15q26.3, 18q23, and 22q13.33 contained *CTDP1, HDAC10, KCNQ1, NINJ2, NOTCH2, PCSK6*, and *SDHA* genes (gained regions), while loss region 7q22.1 contained *MUC17* gene.

Result according to reverse transcriptase quantitative polymerase chain reaction

To confirm the array CGH results, differential fold change was evaluated by RT quantitative PCR in VA samples. The results showed chromosomal fold changes in *CTDP1* (18q23), *HDAC10* (22q13.33), *KCNQ1* (11p15.5-p15.4), *NINJ2* (12p 13.33), *NOTCH2* (1p12-p11.2), *PCSK6* (15q26.3), *SDHA* (5p15.33), and *MUC17* (7q22.1). Fig. 2 shows the RT PCR results. In gained regions of 7 genes, {*CTDP1, HDAC10, KCNQ1, NINJ2, NOTCH2, PCSK6,* and *SDHA* (A-G)}, the relative fold increases in RT quantitative PCR corresponded with array-CGH results. In *MUC17* (H), the decrease in fold differences noted by RT quantitative PCR was similar to that seen with array-CGH. The N-value was delineated in RT PCR. The results compared linear-ratios in array-CGH, were demonstrated generally larger by RT quantitative PCR analysis than those by array-CGH analysis.

Table 2. Primer pairs used for reverse transcriptase quantitative polymerase chain reaction analysis

Genes	Primer forward	Primer reverse	Chromosomal region
Gain			
CTDP1	GTAACGGAGGCTGGTGTGAT	ACTGCTTTGGAAAAGCCTGA	18q23
HDAC10	CACTGTTCACCTGTGGGATG	GCCTGGCTACAGAACGAGAC	22q13.33
KCNQ1	TGCTAGAAGGGGCAGTGAGT	TCAATTTTGGGGATCTCAGC	11p15.5-p15.4
NINJ2	CGCCTGTAATCCCACACTTT	CTCAAGGTCCGGAGTAGCTG	12p13.33
NOTCH2	GGCAACTACAACAGCCCAAT	TCATTTCTGCAGCTGGTCAC	1p12-p11.2
PCSK6	GGCAACAAAAGAAAGCAAGC	TCTCAACAGCTGGGAGAGGT	15q26.3
SDHA	CTGAGTGATGCCCTGCAGTA	TTCCACGAGCAGAGGAAACT	5p15.33
Loss			
MUC17	GCCAGGCTGTTCTTGAACTC	GAAGACGTCCAGACCTGAGC	7q22.1

Table 3. Recurrent gains/losses in spasms {>7 (25%) frequencies}

Chromosomal region	Frequency (%)	Bac_start (bp)	Bac_end (bp)	Size (bp)	Contained genes
Gain					
1p12-p11.2*	11/28 (39)	120390109	120485103	94994	NOTCH2
1p12-p11.2	8/28 (29)	120214296	120390114	175818	ADAM30, NOTCH2
1p12	8/28 (29)	118491301	118584699	93398	SPAG17
1p21.1	8/28 (29)	103961840	103992000	30160	AMY2A
1q44	10/28 (36)	246886515	246967485	80970	OR5BU1
2p11.2	7/28 (25)	86071614	86160853	89239	POLR1A
2q21.2-q21.3	8/28 (29)	134702634	134804417	101783	MGAT5
2q22.1	7/28 (25)	139564813	139702127	137314	
2q23.3	8/28 (29)	150646532	150741461	94929	
2q31.1	8/28 (29)	177310814	177395162	84348	FUCA1P
2q37.3	7/28 (25)	240550837	240713841	163004	NDUFA10, OR6B2, OR6B3, OR5S1P
3p14.1	8/28 (29)	67409363	67499993	90630	
3q12.3	8/28 (29)	103624911	103710584	85673	ZPLD1
3q24	10/28 (36)	149871152	149960984	89832	RPL38P1, AGTR1
4p15.1	11/28 (39)	29961339	30093505	132166	
4p15.1	7/28 (25)	28058051	28175610	117559	
4p15.2	8/28 (29)	26883925	26969030	85105	
4p15.2	8/28 (29)	24962384	25050952	88568	ZCCHC4, ANAPC4
4q24	8/28 (29)	105045356	105165560	120204	
4q32.3	7/28 (25)	167945985	168033177	87192	SPOCK3
4q34.3	8/28 (29)	182485709	182565328	79619	
4q34.3	7/28 (25)	181814958	181921046	106088	
4q35.2	7/28 (25)	190457567	190574280	116713	
4q35.2	8/28 (29)	190712135	190794324	82189	
5p12	8/28 (29)	44338589	44428186	89597	FGF10
5p15.2	8/28 (29)	12789319	12879576	90257	
5p15.33	9/28 (32)	388661	566921	178260	AHRR, EXOC3, SLC9A3
5p15.33*	7/28 (25)	203859	298137	94278	SDHA
5p15.33	14/28 (50)	557250	688780	131530	SLC9A3, CEP72
5q15	7/28 (25)	57292374	57392374	100000	DNHD2
6p12.2	9/28 (32)	51985699	52087584	101885	PKHD1
6q24.1	8/28 (29)	142584814	142703619	118805	GPR126
7q11.23	9/28 (32)	74705985	74764828	58843	
7q22.2	8/28 (29)	105177490	105254527	77037	ATXN7L4
8p23.3	7/28 (25)	1289245	1436212	146967	
8p23.3	8/28 (29)	649638	867290	217652	ERICH1, C8orf68
8q22.2	11/28 (39)	99509647	99629754	120107	KCNS2, STK3
8q24.3	9/28 (32)	145298570	145384455	85885	
9q22.1	7/28 (25)	90613288	90692980	79692	
9q22.32	7/28 (25)	97170096	97284468	114372	PTCH
9q33.3	9/28 (32)	128260025	128347240	87215	C9orf28
10p15.3	9/28 (32)	795549	881206	85657	LARP5
10q11.23	7/28 (25)	52453706	52563182	109476	PRKG1
10q23.1	9/28 (32)	86354131	86459920	105789	
10q26.3	8/28 (29)	133716279	133869047	152768	C10orf39, DPYSL4

Table 3. Continued

Chromosomal region	Frequency (%)	Bac_start (bp)	Bac_end (bp)	Size (bp)	Contained genes
10q26.3	9/28 (32)	134604089	134693987	89898	C10orf93
10q26.3	8/28 (29)	134654530	134754530	100000	GPR123
11p15.5	9/28 (32)	982365	1053559	71194	AP2A2, MUC6
11p15.5-p15.4*	9/28 (32)	2812494	2941798	129304	KCNQ1, KCNQ1DN, CDKN1C, SLC22A18AS, SLC22A18, PHLDA2, NAP1L4
11p15.5-p15.4	8/28 (29)	2759787	2881783	121996	KCNQ1, KCNQ1DN, CDKN1C, SLC22A18AS, SLC22A18
11q12.2	14/28 (50)	60669725	60731709	61984	VPS37C
11q14.1	7/28 (25)	84260432	84364210	103778	DLG2
11q23.1	8/28 (29)	111039892	111136881	96989	SNF1LK2, PPP2R1B
11q23.2	7/28 (25)	114533231	114632257	99026	IGSF4
11q23.3	10/28 (36)	116818967	116903121	84154	
11q25	11/28 (39)	133797141	133894154	97013	
12p13.33	12/28 (43)	120426	213890	93464	IQSEC3, SLC6A12, SLC6A13
12p13.33	13/28 (46)	183679	257363	73684	SLC6A12, SLC6A13
12p13.33*	9/28 (32)	455498	548935	93437	B4GALNT3, NINJ2
12q22	8/28 (29)	92663057	92746083	83026	CRADD
13q33.3	8/28 (29)	107569780	107652035	82255	
14q31.3	8/28 (29)	87706299	87814634	108335	KCNK10
14q32.33	13/28 (46)	105541523	105648959	107436	
15q11.2	13/28 (46)	18881005	18958308	77303	
15q11.2	10/28 (36)	21607011	21719891	112880	
15q25.2	10/28 (36)	80539371	80776796	237425	RPS17
15q26.3	10/28 (36)	100054894	100129431	74537	TARSL2
15q26.3	11/28 (39)	100081440	100155675	74235	TARSL2
15q26.3*	10/28 (36)	99653473	99758817	105344	PCSK6
15q26.3	8/28 (29)	99598506	99716889	118383	CHSY1, SNRPA1, PCSK6
16p11.2	7/28 (25)	32674891	32749246	74355	HERC2P4
16p11.2	8/28 (29)	32022312	32124506	102194	
16p11.2	8/28 (29)	33623082	33716863	93781	
16p12.1	10/28 (36)	75166320	75276261	109941	ATP9B, NFATC1
16p13.2	10/28 (36)	9930139	10035574	105435	GRIN2A
16q24.1	7/28 (25)	84871012	84981407	110395	
17p12	10/28 (36)	14133018	14216943	83925	HS3ST3B1
17q24.3	8/28 (29)	66437827	66557480	119653	
17q25.3	8/28 (29)	78432676	78562724	130048	TBCD, B3GNTL1
17q25.3	7/28 (25)	77755881	77849251	93370	SLC16A3, CSNK1D
18q12.3	10/28 (36)	36570635	36662367	91732	
18q23*	8/28 (29)	75511317	75607690	96373	CTDP1
18q23	7/28 (25)	75349325	75445847	96522	NFATC1
20p12.2	7/28 (25)	10224066	10314894	90828	SNAP25, RPL23AP6
20q12	9/28 (32)	32614251	32731132	116881	ACACA
20q13.33	8/28 (29)	62273049	62364050	91001	MYT1, PCMTD2
21q11.2	10/28 (36)	13725531	13813182	87651	VN1R8P
21q11.2	7/28 (25)	13890255	13992240	101985	ANKRD21
21q22.3	9/28 (32)	42124137	42238168	114031	PRDM15, C21orf25
22q12.2	7/28 (25)	28407057	28496470	89413	NF2, CABP7

Table 3. Continued

Chromosomal region	Frequency (%)	Bac_start (bp)	Bac_end (bp)	Size (bp)	Contained genes
22q13.33*	9/28 (32)	48930979	49068912	137933	MOV10L1, PANX2, TUBGCP6, HDAC10, MAPK12, MAPK11, PLXNB2
Loss					
2p24.1	7/28 (25)	20200860	20294981	94121	SDC1
4q35.2	12/28 (43)	190963818	191006683	42865	
7q22.1*	15/28 (54)	100407386	100480418	73032	MUC12, MUC17
7q36.3	7/28 (25)	157803062	157924916	121854	PTPRN2
10q26.3	9/28 (32)	134755203	134904547	149344	GPR123, KNDC1, UTF1, VENTX
10q26.3	10/28 (36)	134442073	134556094	114021	INPP5A, NKX6-2, C10orf92
15q11.2	11/28 (39)	19958100	20053879	95779	VSIG6
16p13.11	9/28 (32)	16274784	16391850	117066	NOMO3, PKD1P1, PKD1P2
16p13.11	8/28 (29)	14873336	14958740	85404	NOMO1, PKD1P3, NPIP
16p13.3	7/28 (25)	2066833	2157921	91088	TSC2, PKD1, RAB26, TRAF7
17p11.2	9/28 (32)	21826617	21845534	18917	
19p13.3	9/28 (32)	292986	371099	78113	MIER2, THEG, KIAA1957

*Verified by reverse transcriptase polymerase chain reaction

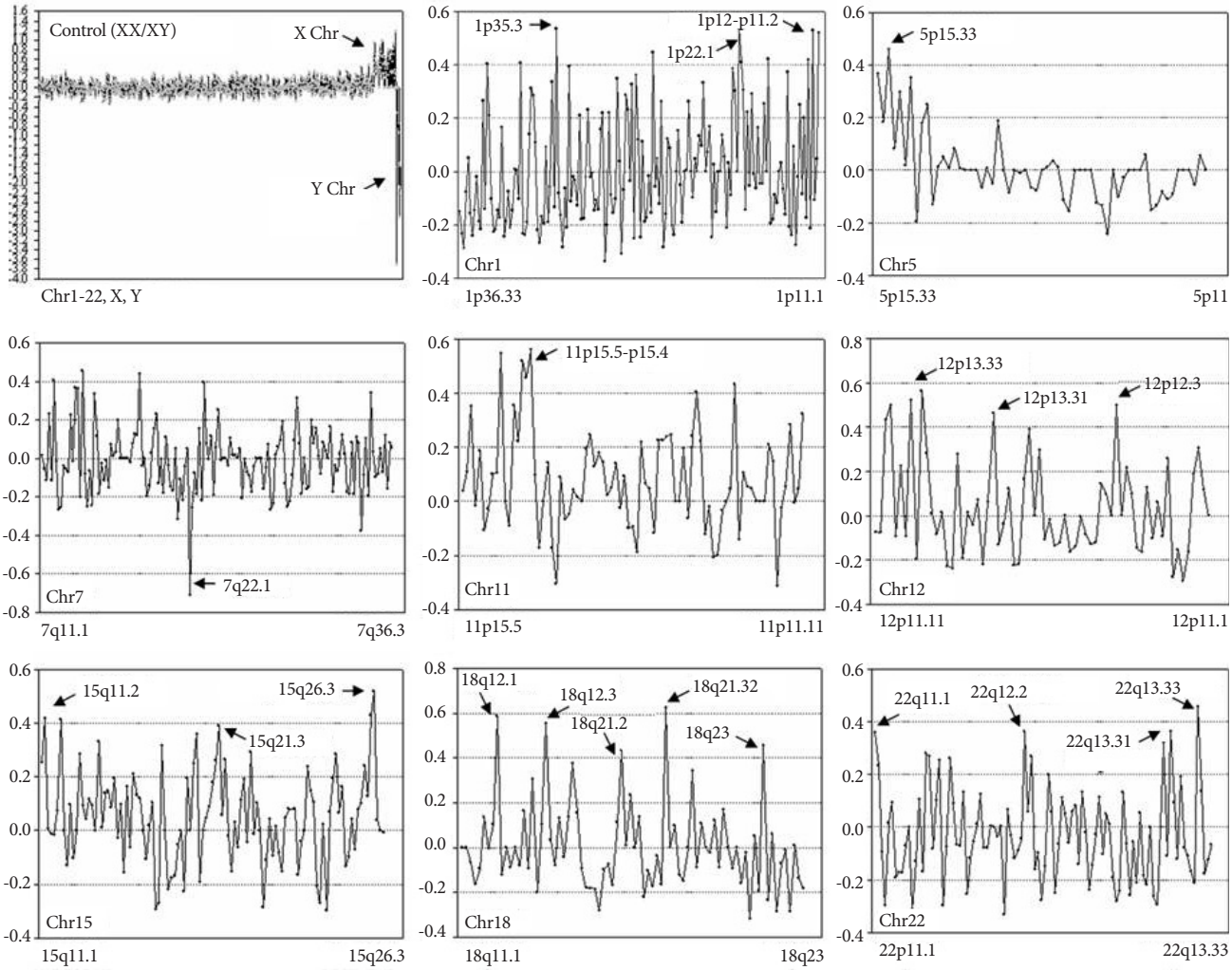

Fig. 1. Array comparative genomic hybridization (CGH) results. Average $\log_2$ ratios (Y axis) of each chromosomal region at 1p, 5p, 7q, 11p, 12p, 15q, 18q and 22q (X axis) show differences in copy number in spasm patients. Internal control (XX/XY) was used to detect genomic imbalances. Note the high-level aberrations (arrowed spots) on chromosomes.

Discussion

Several methods including classical cytogenetics, FISH, Southern blot analysis, metaphase CGH, and array CGH have been used to detect DNA copy number changes.[23] Lately, array CGH has been shown to be a powerful tool for detecting genetic aberrations and genetic mapping, and offers high resolution, high-throughput, accuracy, and sensitivity for genetic analysis.[12-14] It also allows the simultaneous quantitative analysis of all regions of a large genome,[25] and can pro-

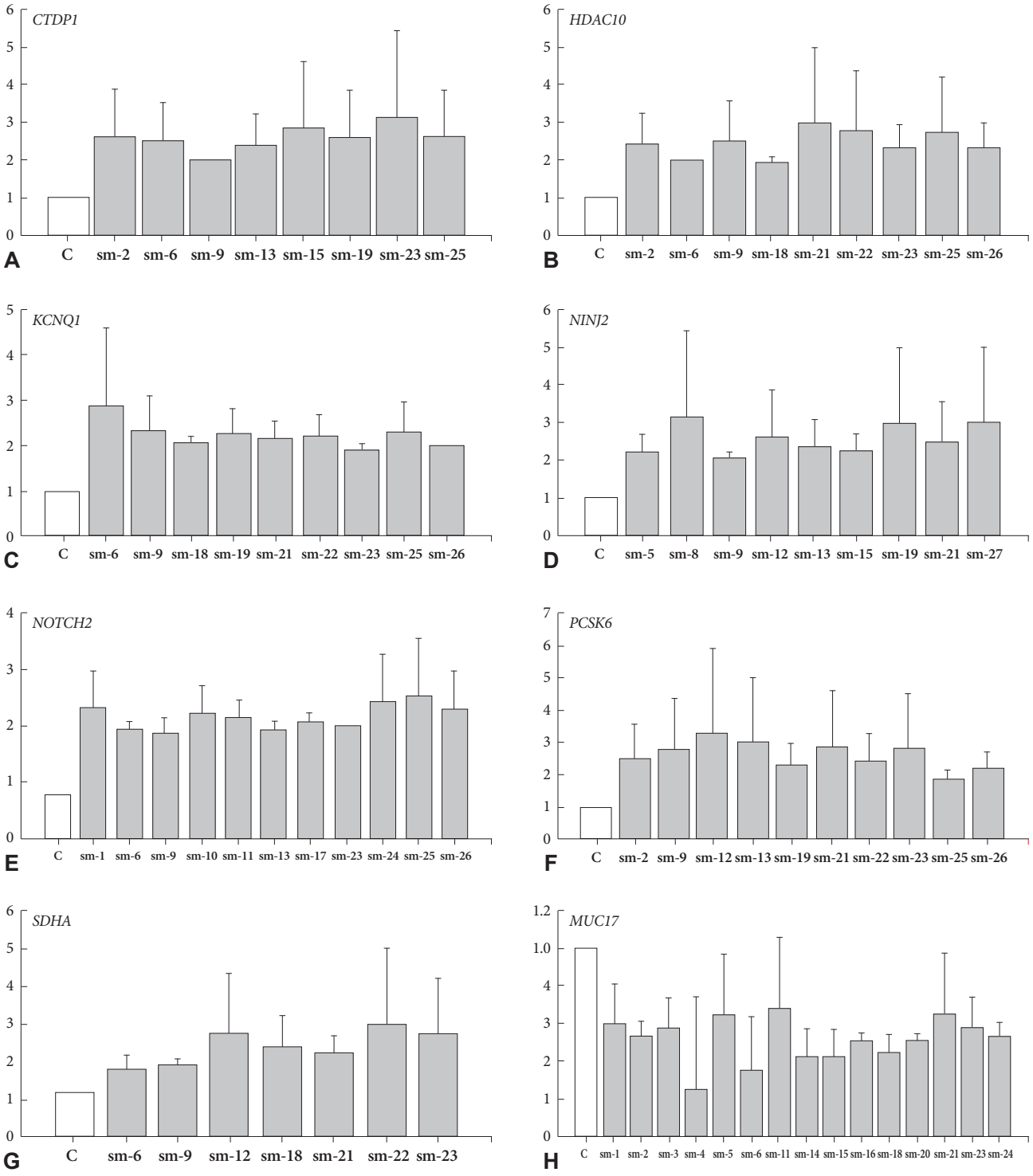

Fig. 2. Relative fold differences selected from 8 genes showed that the most frequent gains and losses detected were at 1p, 5p, 7q, 11p, 12p, 15q, 18q and 22q region. Each sample is depicted (X axis) and the fold difference of N-value was delineated in RT PCR (Y axis). A threshold level of 2 (N-value) indicates significant DNA gain (A-G), and 0.5 (N-value) indicates significant DNA loss (H). RT: reverse transcriptase, PCR: polymerase chain reaction, DNA: deoxyribonucleic acid.

vide quantitative information at the level of chromosomal gain or loss.[21]

Although the confirmation of array-CGH results is usually performed by FISH analysis, this technique has several limitations. For example, evaluation of the full extent of genetic gains or losses in the genome requires obtaining metaphase DNA and interpretion of karyotypes that are often complex and require prior knowledge or markers of sites of interest.[2] Many recent studies have shown that RT quantitative PCR instead of FISH is also a very useful and accurate technique for the confirmation of array-CGH results,[17][18][21-24] thus making it particularly attractive technique for the identification of acquired genetic aberrations in whole blood from patients with VA. Therefore, we used RT quantitative PCR for the confirmation and quantification of the identified genomic aberrations.

In this study, using array CGH, we have showed than there are genetic aberrations and altered genes related to VA. The DNA aberrations had a tendency to be located at rather specific chromosomal regions. Ulike amplification of oncogenes or deletions of tumor suppressor genes[21-24] there were no specific genes amplified or deleted in VA in our study. However, many DNA copy number aberrations (91 DNA gains and 12 DNA losses) were detected. These DNA copy number aberrations were novel and different from those previously detected in the Korean population (DNA gains in 6p21.2 and DNA loses in 1p36.31, 4q13.1-4q22.13, 16p12.1, 21q22.3, and 22q11.22).[31] This showed that many genetic alterations might be associated with the pathogenesis of VA.

Because array CGH shows genetic aberrations at the genomic DNA level and not at the messenger RNA or protein levels, many chromosomal regions which might be related to the pathogenesis of VA were found in this study. For example, the *KCNQ1* gene, one of the genes analyzed by RT quantitative PCR,[27] is translated into the potassium voltage-gated channel subfamily KQT member1, which is probably related to cardiac repolarization (www.ihop-net.org/UniPub/iHOP). Also, the *NOTCH2* gene, another one of the genes analyzed by RT quantitative PCR, is translated into neurogenic locus notch homolog protein 2 precursors; notch homolog protein 2 is a receptor for the membrane-bound ligands involved in regulation of cell-fate determination (www.ihop-net.org/UniPub/iHOP). However, the function of the proteins translated by these genes has not been completely clarified, so definite pathogenesis of VA cannot be established based on our results. Nevertheless, our data may become the cornerstone that will establish the function of each genetic aberration involved in the pathogenesis of VA.

Conclusion

In summary, using array CGH, we analyzed genome-wide chromosomal aberrations and screened chromosomal candidate regions related to the pathogenesis of VA. The array-CGH results were confirmed by RT quantitative PCR. This study is the first for searching whole genomic alterations by array CGH in patients with VA. Our study may contribute to further clarification of important chromosomal regions, identification of candidate genes, and understanding of the pathogenesis of VA.

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