19 例、se(a2)；10 例、si；1 例。

c. リンパ節転移別病巣数：n(-)：
32 例、n1(+)：18 例 n2(+)：
2 例,n3(+)：0 例、不明：3 例

d. 根治度別症例数：根治度 A：43 例、
根治度 B：11 例、根治度 C：1 例

2）手術成績

a. 手術術式
前方切除；43 例（腹腔鏡下；34 例、
開腹下； 9 例）
腹会陰式・腹仙骨式直腸切断術；6 例
（腹腔鏡下；1 例、開腹下；5 例）
ハルトマン手術；1 例
経肛門的局所切除術；5 例

b. 手術時間
前方切除；227±35 分
腹会陰式・腹仙骨式直腸切断術；
245±23 分

c. 出血量
前方切除； 151±80g
腹会陰式・腹仙骨式直腸切断術； 283±73g

3）術後短期予後

a. 術後合併症
縫合不全；4 例
吻合部出血；1 例
創感染（会陰創含む）；3 例
排尿障害；2 例
イレウス；2 例
その他；1 例

5）当院における登録の実績

a.平成 18 年度の当科における適格症例数：
9 例

b.同意説明施行患者：6 例
同意説明非施行理由：
肛門温存を含めて術前化学放射線
治療を薦めた-3 例

c.同意取得患者-2 例

d.同意非取得患者-4 例
理由：標準（側方郭清）手術を希望-
4 例　TME を希望-0 例

D. 考察

当科では進行直腸癌に対する手術においては、基本的には側方郭清を追加する方針である。今回 JCOG の臨床試験に参加して後もその方針に変わりはない。そこで、適格患者に対して臨床試験の IC を行う際には、当科の基本指針を説明した後に、海外からの Evidence を示しながら TME のみの手術の利点を説明している。これにより、IC 取得率は 2/6（33.3%）と低率で、患者の拒否理由を見るとすべて側方郭清手術の希望であった。日本の標準治療が側方郭清である現状から多少の手術侵襲の付加と合併症の増加があってもこれを希望するという現状との理解である。

一方近年肛門温存を含めた機能温存手術も提唱され、また、術前の放射線療法も勧められてきた中で、当科においても肛門括約筋機能が比較的良く保たれている患者さんや、放射線療法に関して理解のある患者さんに対しては術前化学放射線療法の説明もしている。これにより若干適格患者数が減少している部分もある。今後この分野の占める割合も増えることが予想され、側方郭清追加の有無をみる試験にのみ登録することが難しいという面もある。しかし積極的に適格患者が発生すれば IC を取るという方針に変わりはなく今後

もこれを続行していきたい。

E. 結論

当科における進行直腸癌に対する手術において、JCOG 試験に参加した。術前化学放射線療法も必要なためすべての患者にIC説明も難しいが、その他の患者においては IC 取得率の向上を目指す。

F. 健康危険情報

なし

G. 研究発表

1．論文発表

なし

2． 学会発表

なし

H. 知的財産権の出願・登録状況

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

厚生労働科学研究費補助金（がん臨床研究事業）

分担研究報告書

側方リンパ節郭清術の意義に関するランダム化比較試験に関する研究

分担研究者　冨田　尚裕　関西労災病院　外科第二部長

研究要旨 臨床病期II,IIIの下部直腸癌に対する神経温存D3郭清術の意義に関するランダム化比較試験(JCOG0212(ME vs. ANP-D3))に共同研究参加施設として参加している。昨年の報告時点（平成１８年２月）からは現在のところ、追加の症例登録はないが、今までの登録症例に関してはフォローアップ中も特に重篤な有害事象や脱落症例を認めず、今後も本試験登録の促進と共に、登録症例の予後等についての追跡調査を継続する予定である。

A．研究目的

臨床病期II,IIIの下部直腸癌に対する神経温存D3郭清術の意義に関するランダム化比較試験(JCOG0212(ME vs. ANP-D3))に共同研究参加施設として参加し、プロトコール治療を行い、進行下部直腸癌の手術における側方リンパ節郭清の意義を検証するためのデータを得ることを目的とした。

B．研究方法

当院での進行下部直腸癌手術症例において、JCOG0212のプロトコールに定められたエントリー基準に従って術前に症例を選択し（Informed Consentのもと）、術中所見に従ってプロトコール通りにA,B2群にランダム割付を行い、それぞれプロトコール通りに手術を行い、術後化学療法の有無、検査などもプロトコール通りに決定し、遂行する。登録症例について有害事象、予後などの調査を行う。研究方法の詳細はプロトコール通りである。

C．研究結果

昨年の報告時点（平成１８年２月）までに４例の登録を行い、特に重篤な有害事象や脱落症例は認めなかった。それ以降、現在まで追加の症例登録は無いが、今までの登録症例に関してはフォローアップ中も特に重篤な有害事象や脱落症例を認めていない。

D．考察

現時点で特に本研究の継続には問題は無く、今後、予後などのデータの蓄積を待って考察を行っていく予定である。

E．結論

今後も予後等の追跡調査を行っていく予定である。

F．研究発表

1．論文発表

なし

２．学会発表

なし

（発表誌名巻号・頁・発行年等も記入）

G．知的所有権の取得状況

１．特許取得

なし

２．実用新案登録

なし

３．その他

なし

厚生労働科学研究費補助金（がん臨床研究事業）

分担研究報告書

側方リンパ節郭清術の意義に関するランダム化比較試験に関する研究

分担研究者　赤在　義浩　　岡山済生会総合病院　外科主任医長

研究要旨　多施設共同研究 JCOG０２１２試験に参加して、下部直腸がんに対する側方リンパ節郭清の意義を検討するため、症例登録中である

A. 研究目的

術前画像診断および術中開腹所見にて、明らかな側方骨盤リンパ節転移を認めない臨床病期 II・III の治癒切除可能な下部直腸がん患者を対象として、mesorectal excision（ME 単独）と自律神経温存 D3 郭清術（神経温存 D3 郭清）の臨床的有用性を比較評価する。

B. 研究方法

術前画像診断にて登録適格規準を満たした症例に、インフォームドコンセントを行い同意取得後、術中開腹所見を確認し、中央割付法で２群にランダム化する。

（倫理面への配慮）

院内 IRB の承認を得た。

C. 研究成果

現在登録中であり、当院より１３症例の登録を行った。男性が１０例と女性が３例で、神経温存 D3 郭清が７例と ME 単独が６例であった。登録１３症例のうちリンパ節転移は４例に認めたが、神経温存 D3 郭清７例のうちリンパ節転移は２例のみで側方リンパ節転移は認めなかった。神経温存 D3 郭清７例を含む登録１３症例全員に術後の排尿障害は認めなかった。術前の性機能アンケート調査は男性１０例全員に行い、術後１年経過後の性機能アンケート調査も１年経過７例全員に行った。今後も未調査の無いように、順次１年経過後の外来受診時に依頼予定である。

登録１３症例のうち再発を３例に認めた。再発３例はともに神経温存 D3 郭清群で、肝転移が２例と、大動脈周囲リンパ節転移が１例であった。再発３例の初回手術時リンパ節転移は、肝転移２例はともに n0、大動脈周囲リンパ節転移例は n2 であった。再発３例はともに再切除術が施行された。

D. 考察

登録は１３症例と少数であるが、神経温存 D3 郭清７例と ME 単独６例の術後早期合併症に差はなく、排尿障害は両群とも認めなかった。さらに症例を集積して両群の有用性を比較評価する必要があると考えられる。

E. 結論

本試験は有意義であり、今後も継続すべきである。

F. 研究発表

なし。

G. 知的所有権の取得状況

なし

厚生労働科学研究費補助金（がん臨床研究事業）
分担研究報告書
側方リンパ節郭清術の意義に関するランダム化比較試験に関する研究

分担研究者　分担研究者　　白水　和雄　　久留米大学医学部外科　教授

研究要旨　明らかな側方骨盤リンパ節転移を認めない臨床病期II・IIIの治癒切除可能な下部直腸癌患者を対象とし、国内標準手術である自律神経温存D3郭清術（神経温存D3郭清）の臨床的有用性を、国際標準手術のmesorectal excision（ME単独）を対照とした多施設共同臨床試験にて評価する。当施設では現在までに7例を登録した。ME単独群の1例に術後縫合不全を合併したが、再発は認めていない。引き続き症例の登録を行う予定である

A．研究目的

欧米では、下部直腸癌に対し mesorectal excision（ME）が標準術式とされている。本邦では、ある一定の確率で側方骨盤リンパ節転移が存在することから自律神経機能を維持しつつ側方リンパ節郭清を施行している。しかし、側方リンパ節郭清の明らかなエビデンスはなく、その意義については不明である。そこで、明らかな側方骨盤リンパ節転移を認めない臨床病期 II・III の治癒切除可能な下部直腸癌患者を対象とし、国内標準手術である自律神経温存 D3 郭清術（神経温存 D3 郭清）の臨床的有用性を、国際標準手術の mesorectal excision（ME 単独）を対照とした多施設共同ランダム化比較試験にて評価する。

B．研究方法

（対象）

臨床病期が II 期または III 期の腫瘍下縁が腹膜翻転部と肛門縁に存在する下部直腸癌。年齢が 20 歳から 75 歳までの PS 0-1 で、mesorectum 外にリンパ節転移および浸潤が無い症例。

（エンドポイント）

Primary endpoint: 無再発生存期間

Secondary endpoint: 生存期間、局所無再発生存期間、有害事象発生率、手術時間、出血量、性機能障害発生率、排尿機能障害発生率

（治療）

A 群：ME＋神経温存 D3 郭清

B 群：ME

p-stage III の場合、術後補助化学療法 5-FU+l-LV（8 週 1 コース×3 コース）施行

（割付調整因子）

術中リンパ節転移の有無、性別、施設

（予定症例数、登録期間、追跡期間）

600 例、登録期間 5 年（2003 年 6 月より開始）、追跡期間 5 年

B．倫理面への配慮

すべての研究者はヘルシンキ宣言に従って本試験を実施する。十分な説明と同意を得る（インフォームドコンセント）。登録患者の氏名は試験データセンターへ知らせることはなく、登録者の同定や照会は、登録時に発行される症例登録番号、患者イニシャル、生年月日、カルテ番号を用いて行われ，患者名など第三者が直接患者を識別できる情報がデータセンターのデータベースに登録されることはない。本試験に参加する研究者は，患者の安全と人権を損なわない限りにおいて本研究実施計画書を遵守する．有害事象の発生に対しては保険診療の範囲で適切かつ迅速な対応をとる。

C．研究結果

現在までに、7 例を登録した。内訳は A 群 4

例、B 群 3 例であった。B 群の 1 例に縫合不全を合併したが、保存的に治療可能であった。その他特記すべき有害事象の発生はなかった。登録症例数が少数な理由として、比較臨床試験における患者さんの試験参加同意が得にくいことがあげられた。

D. 考察

比較臨床試験への参加同意を得られない患者が多かった。したがって、当施設の予定登録数を大幅に下回った。全体の登録数についても予定を下まわっている。臨床試験、とくに比較臨床試験の重要性を医療提供者および患者双方が認識することが肝要であり、そのための啓発活動も重要であると思われる。

E. 結論

試験デザインは適正と思われる。予定期間中にできるだけ多くの症例の登録が必要である。

F. 健康危険情報

特記なし

G. 研究発表

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H. 知的財産権の出願・登録状況

1．特許取得

なし

2．実用新案登録

なし

III. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

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IV. 研究成果の刊行物・印刷

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Allelic Status of Chromosomes 17p, 18q, 22q, 3p and their Clinical Usefulness in Colorectal Cancer

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Abstract. *Background: To determine whether the allelic status of chromosomes is clinically useful in colorectal cancer, the allelic losses at chromosomes 17p, 18q, 22q and 3p and their relationships with the clinicopathological features in colorectal cancer (CRC) patients, who had undergone curative surgery without adjuvant chemotherapy, were examined. Materials and Methods: The allelic status at 17p, 18q, 22q and 3p was analyzed by PCR-SSCP (polymerase chain reaction single-strand conformation polymorphism) in 139 CRC from patients who had undergone curative surgery between October 1994 and June 1996. The relationships between these allelic losses and the clinicopathological features were investigated. Results: The lymph node status was significantly associated with the allelic status of 17p, 18q and 22q. The tumor site and tumor differentiation were significantly associated with the allelic status of 18q. When patients with more than two allelic losses were defined as the high allelic loss group and those with no, or only one allelic loss were defined as the low allelic loss group, it was found that the lymph node involvement was significantly higher in the high than in the low allelic loss group. Only three out of 25 patients in the low allelic loss group had lymph node metastasis, and 15 patients in this group without lymphatic invasion had no lymph node metastasis. There was no relationship between the allelic status and survival at any stage. Conclusion: The allelic status was significantly associated with lymph node metastasis. A combination of allelic status and lymphatic invasion assessment can predict the lymph node status before radical surgery.*

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Key Words: Colorectal cancer, allelic status, lymphatic invasion, lymph node status.

There have been many reports on the relationships between the clinicopathological features of colorectal cancer (CRC) patients and the allelic status of chromosomes 1p (1, 2), 2p (3), 3p (4), 4p (5), 5q (6), 8p (7, 8), 17p (1, 8, 9) and 18q (8-15), or a combination of different allelic statuses (8, 16). Several reports have shown that the prognosis for patients with allelic losses is worse than for those without allelic losses. However, there have been conflicting results for chromosomes 5q (6), 17p (15, 17-19) and 18q (1, 17, 19, 20) and for combinations of chromosomal alterations (21). Therefore, these genetic alterations of allelic status are not clinically used for CRC.

To determine whether the allelic status is, in fact, clinically useful in CRC, four chromosomes were studied: 17p, 18q, 22q and 3p. Chromosomes 17p and 18q have tumor suppressor genes, p53 and DCC, respectively, and their allelic status has been suggested, in many reports, to be associated with clinicopathological features (1, 8, 10-15). The allelic loss of 22q is relatively frequent in CRC (22-24), but there have been no reports of a relationship between the clinical background and the allelic status of 22q. The allelic status of 3p was reported to be associated with survival prognosis (4), and preferential allelic loss of 3p was observed in metastatic tumors in comparison with primary CRC (25). The status of these four chromosomes in 139 cancers, obtained from CRC patients who had undergone curative surgery without adjuvant chemotherapy, was analyzed. Then, the relationship between the allelic status and clinicopathological features was examined.

Materials and Methods

Patients and tissues. A total of 139 CRC, from patients who had undergone curative surgery without adjuvant chemotherapy at the National Cancer Hospital, Tokyo, Japan, between October 1994 and June 1996, were examined. The primary tumors had been obtained immediately after surgery and stored frozen in liquid nitrogen until DNA extraction. All surviving patients had been followed for more than 5 years, initially at 3-month intervals for

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2 years and at 6-month intervals thereafter. Adjuvant chemotherapy had not been given.

Blunt-end SSCP analysis of allelic status. The allelic status was determined by blunt-end SSCP (single-strand conformation polymorphism) analysis (26, 27). Briefly, three intragenic polymorphic markers (intron 1, exon 4 and intron 7) of the *p53* gene, and two 17p13 markers (D17S695, D17S919), a 17p11 marker (D17S969), two 18q21 markers (D18S51, D18S499), a 22q12 marker (D22S685) and a 3p23 marker (D3S2396) were analyzed by blunt-end SSCP. For the amplification of these polymorphic markers, the primers shown in Table I were used. The forward and reverse primers were synthesized and labelled with indodicarbocyanine (Cy5) amidite reagent, a fluorescent dye (Pharmacia, Uppsala, Sweden), using an Oligo 1000 DNA synthesizer (Beckman, Fullerton, CA, USA). In PCR, the first denaturation step was done at 95°C for 5 min. PCR amplification was performed for 30 to 40 cycles under the following conditions: denaturation at 95°C for 30 to 60 sec, annealing at 50 to 67°C for 30 to 60 sec and extension at 72°C for 30 to 60 sec. For the blunting reaction, 0.5 units of Klenow fragment (TAKARA BIO, Shiga, Japan) was added to 5 μL of the PCR product and incubated at 37°C for 30 min. One microliter of the reaction mixture was mixed with 10 μL of the loading buffer and denatured at 80°C for 5 min. One microliter of the aliquot was electrophoresed on 15% polyacrylamide gel at 20°C to 24°C for 10 h at 20 W using an ALFred DNA sequencer (Pharmacia). The data were analyzed using the Fragment Manager (Pharmacia) software package. In the analysis of a normal heterozygote, the ratio of the peak heights of the signal from each allele was constant, with a variation of within 5% (27). Therefore, an allelic loss was defined as when one of the peak heights for a tumor sample was decreased by more than 10% of that of the corresponding normal tissue. Supposing the A1 allele is lost in a heterozygote carrying the A1 and A2 alleles, T is the peak height of the signal from the tumor samples, and N is the peak height of the signal from the normal control. Then, the percent peak height (%) is given as:

$$(N_{A1}/N_{A2} - T_{A1}/T_{A2}) \times 100 / (N_{A1}/N_{A2})$$ (26).

If at least one of the markers of the same chromosome showed an allelic loss, the chromosome was defined as having an allelic loss.

Statistical analysis. Statistical analysis was carried out by the Chi-squared test. The survival rates were calculated by the Kaplan-Meier method and survival curves were compared by the log-rank test. Cox's proportional hazard model was used for multivariate analysis. The level of statistical significance was set at <0.05.

Table I. *Primers used for PCR-SSCP analysis.*

Forward	Reverse
17p11-13	
D17S695	
5'CTGGGCAACAAG AGCAAAATTC3'	5'TTTGTTGTTGTTCAT TGACTTCAGTCT3'
D17S919	
5'AGGCACAGAGT GAGACTTG3'	5'GCTTAATTTTCACGA GGTTCAG3'
p53 intron 1	
5'TCTTAGCTCGCG GTTGTTTC3'	5'ACTGGCGCTGTGT GTAAATG3'
p53 exon 4	
5'AGCTCCCAGAAT GCGAGAG3'	5'CTGGGAAGGGACA GAAGATG3'
p53 intron7	
5'AGGTCAGGAGCC ACTTGCC3'	5' GTGATGAGAGGTG GATGGGT3'
D17S969	
5'ATCTAATCTGTCA TTCATCTATCCA3'	5'AACTGCAGTGCTG CATCATA3'
18q21	
D18S51	
5'GAGCCATGTTCA TGCCACTG3'	5'CAAACCCGACTAC CAGCAAC3'
D18S499	
5'CTGCACAACATA GTGAGACCTG3'	5'AGATTACCCAGAA ATGAGATCAGC3'
22q12	
D22S685	
5'TTCTTAGTGGGGA AGGGATC3'	5'TGAGTTTGATGTTT TTGATAGACA3'
3p23	
D3S2396	
5'ACCTCTTACTTGT GTTCTTGGG3'	5'TGACCAAGCC AGTATTGGAT3'

Results

Allelic status and clinicopathological backgrounds. The allelic status of 17p was informative in all the patients, the allelic status of 18q was informative in 136 patients (98%), that of 22q was informative in 122 patients (88%) and that of 3p was informative in 106 patients (76%). Representative electropherogram profiles from the SSCP analyses are shown in Figure 1. The clinicopathological backgrounds of the informative cases are shown in Table II. The lymph node status was significantly associated with the allelic status of 17p, 18q and 22q ($p<0.01$, <0.01 and 0.01, respectively). The tumor site and tumor differentiation were significantly associated with the allelic status of 18q ($p<0.01$ and 0.03, respectively). To examine the relationships between the number of allelic losses and the clinicopathological backgrounds, the examined patients were classified into high and low allelic loss groups. The high allelic loss group contained patients with more than two allelic losses. The low allelic loss group contained patients with no, or only one allelic loss. Patients with more than two non-informative alleles or with one allelic loss and one non-informative allele were excluded, because these patients' allelic status could not be classified into either group. In this way

Table II. *Clinicopathological backgrounds for informative cases.*

Chromosomes	17p		18q		22q		3p	
	Loss	Retained	Loss	Retained	Loss	Retained	Loss	Retained
Gender								
Male	68	16	68	15	41	31	28	37
Female	38	17	41	12	27	23	15	26
p	0.11		0.51		0.75		0.51	
Age								
<60	40	11	43	8	23	20	16	23
60≤	66	22	66	19	45	34	27	40
p	0.65		0.35		0.71		0.94	
Tumor site								
Colon	63	25	63	23	42	38	30	39
Rectum	43	8	46	4	26	16	13	24
p	0.09		< 0.01		0.32		0.40	
Tumor differentiation								
Well	46	20	47	18	38	24	19	26
Moderate	60	13	62	9	30	30	24	37
p	0.08		0.03		0.21		0.77	
Lymphatic invasion								
Negative	47	19	50	14	30	31	17	31
Positive	59	14	59	13	38	23	26	32
p	0.18		0.58		0.14		0.33	
Venous invasion								
Negative	56	22	57	19	39	32	24	33
Positive	50	11	52	8	29	22	19	30
p	0.16		0.09		0.83		0.73	
Depth of invasion (pT)								
pT1, pT2	18	10	23	5	11	13	9	14
pT3, pT4	88	23	86	22	57	41	34	49
p	0.10		0.77		0.28		0.87	
Lymph node status (pN)								
Negative	49	25	49	22	31	37	22	34
Positive	57	8	60	5	37	17	21	29
p	<0.01		<0.01		0.01		0.77	

seven patients were excluded. The clinicopathological backgrounds of patients in the high and low allelic loss groups are shown in Table III. The lymph node status was significantly associated with high and low allelic status ($p<0.01$). In the low allelic loss group, only three CRC patients out of 25 (12%) patients had lymph node metastases, while 15 patients without lymphatic invasion had no lymph node metastasis.

Allelic status and disease-free survival. The disease-free survival rates are shown in Table IV. In stages I and II, the high allelic loss group showed slightly worse survival than the low allelic loss group. In stage III, patients with allelic loss at 18q showed worse survival than those without allelic loss at 18q, and the high allelic loss group also showed worse survival than the low allelic loss group (Figure 2). Patients with allelic loss at 3p and those with allelic loss at 22q showed better survival than those without these allelic losses. However, these differences were not significant. In multivariate analysis, only the lymph node status was selected as a significant prognostic factor.

Discussion

Many reports have shown relationships between the clinicopathological background or prognosis of CRC patients and their allelic status (1-16). However, these allelic status relationships are not used clinically because the results have not been fully validated. Of the four chromosomes examined here, allelic loss at chromosome 18q has been suggested to have a strong association with poor prognosis for CRC patients in many reports (8-15). However, some reports, including our study, did not show a significant association between the allelic status of 18q and prognosis (1, 17, 19, 20). Barratt *et al.* suggested that there was an interaction between the allelic status and response to adjuvant therapy (19). Their results showed that only patients without allelic loss gained survival benefits from adjuvant therapy, while those with allelic loss did not. This explains the conflicting results of the association between allelic status and prognosis, because many studies into allelic status included patients who either did or did not receive

A

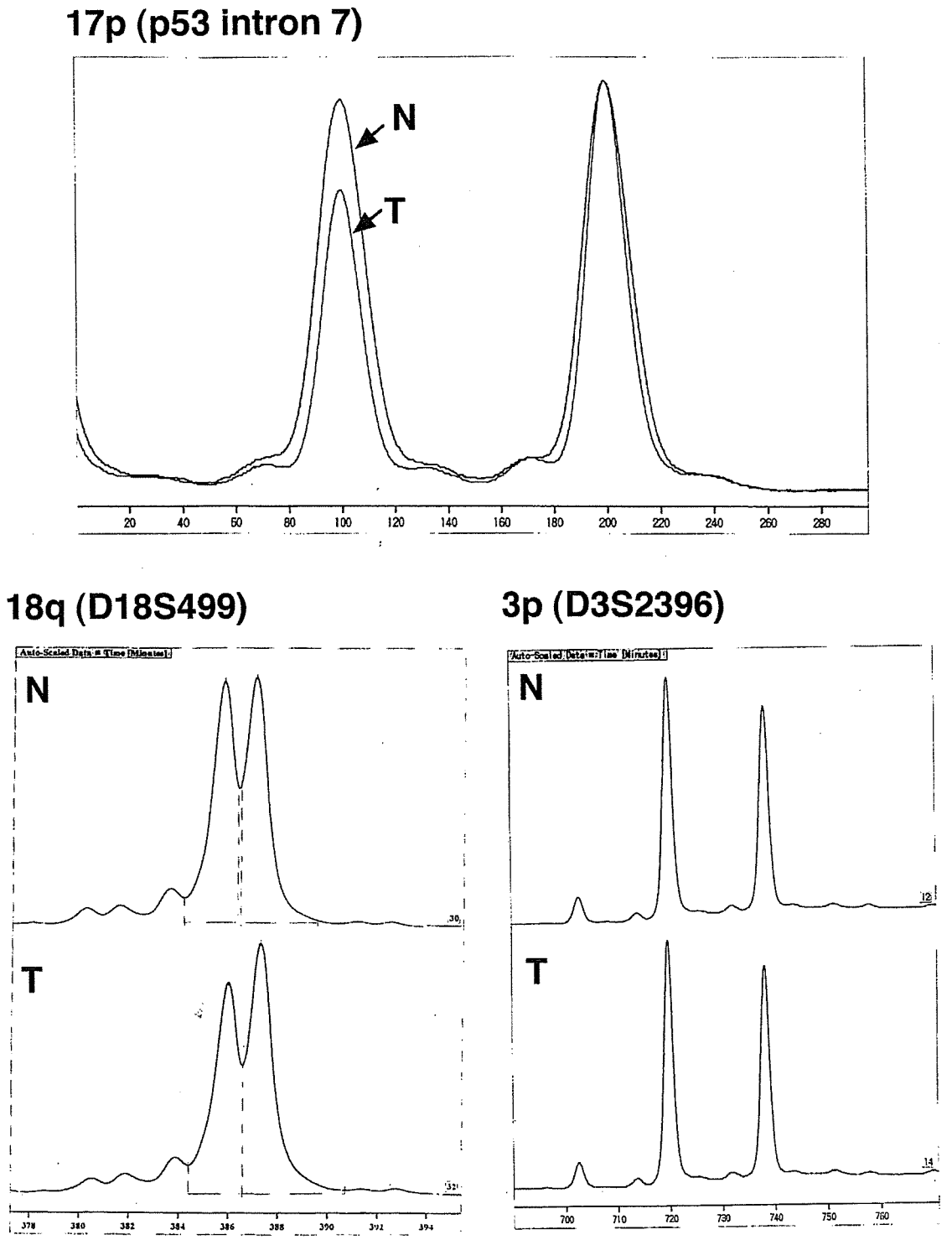
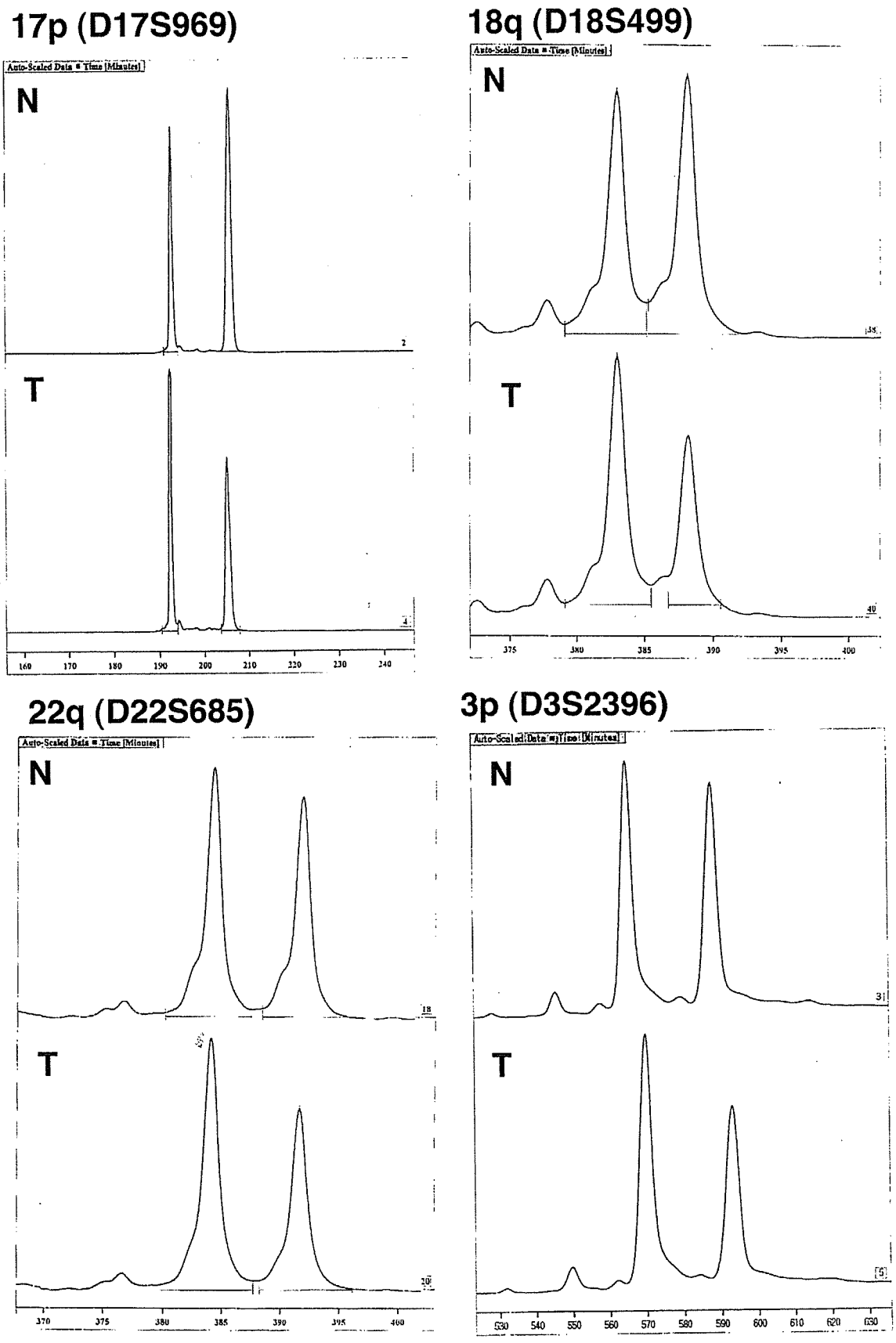


Figure 1. *Electropherogram profiles in SSCP analysis. A: Percent peak height of tumor tissue profile was 23%, 14% and 1% at p53 intron 7, D18S499 and D3S2396, respectively. As defined in Materials and Methods, this patient had allelic loss of 17p and 18q, while the allele of 3p was retained. The allele of 22q was not informative (data not shown). B: Percent peak height of the tumor tissue profile was 43%, 36%, 18% and 22% at D17S969, D18S499 D22S685 and D3S2396, respectively. All the alleles examined were lost in this patient.*

B


17p (D17S969)
Auto-Scaled Data = Time [Minutes]
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18q (D18S499)
Auto-Scaled Data = Time [Minutes]
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375 380 385 390 395 400
22q (D22S685)
Auto-Scaled Data = Time [Minutes]
N
T
370 375 380 385 390 395 400
3p (D3S2396)
Auto-Scaled Data = Time [Minutes]
N
T
530 540 550 560 570 580 590 600 610 620 630

2837

Table III. *Clinicopathological backgrounds of low and high allelic loss groups.*

Allelic loss	Low (n=25)	High (n=107)	*p*
Gender			
Male	12	68	
Female	13	39	0.29
Age			
<60	8	40	
60≤	17	67	0.61
Tumor site			
Colon	19	63	
Rectum	6	44	0.11
Tumor differentiation			
Well	15	47	
Moderate	10	60	0.15
Lymphatic invasion			
Negative	15	49	
Positive	10	58	0.20
Venous invasion			
Negative	17	57	
Positive	8	50	0.18
Depth of invasion (pT)			
pT1, pT2	7	19	
pT3, pT4	18	88	0.25
Lymph node status (pN)			
Negative	22	47	
Positive	3	60	<0.01

Table IV. *Disease-free survival according to allelic status.*

		5-year disease-free survival rate			
		Stage I, II	*p*	Stage III	*p*
17p	Loss	80% (n=49)		56% (n=57)	
	Retained	80% (n=25)	0.96	63% (n=8)	0.80
18q	Loss	81% (n=49)		54% (n=60)	
	Retained	85% (n=22)	0.62	71% (n=5)	0.34
22q	Loss	83% (n=31)		68% (n=37)	
	Retained	83% (n=37)	0.79	47% (n=17)	0.19
3p	Loss	82% (n=22)		67% (n=21)	
	Retained	81% (n=34)	0.79	45% (n=29)	0.36
High and low allelic loss status					
	High	77% (n=47)		59% (n=60)	
	Low	86% (n=22)	0.30	67% (n=3)	0.83

adjuvant therapy. Another explanation for the conflicting data is non-specific allelic loss. Because chromosomal losses and gains are driven by chromosomal instability that persists throughout the lifetime of the tumor cells (28), some of the allelic losses may not affect the malignant potential of cancer cells, and these non-specific alterations may decrease the prognostic importance of the allelic losses, *i.e.*, these non-specific alterations may obscure the effects of allelic loss.

We showed that the allelic status was significantly related to the lymph node status. If the lymph node status could be predicted before radical surgery, it would be useful for clinical decision making. Taking the high-risk factor for lymph node metastasis, lymphatic invasion (29, 30), into account, patients without allelic loss and without lymphatic invasion had a very low incidence of lymph node metastasis. Among 14 patients without allelic loss at 18q or lymphatic invasion, only one patient (7%) had lymph node metastasis. Among 19 patients without allelic loss at 17p or lymphatic invasion, only one patient (5%) had lymph node metastasis. Fifteen patients in the low allelic loss group without lymphatic invasion had no lymph node metastasis. However, the presence of lymphatic invasion cannot be determined before resection, only after. These results suggested that the combination of allelic loss status and lymphatic invasion status can predict lymph node metastasis before radical surgery. This is particularly useful information, especially for T2 or more so for rectal cancer because, *e.g.*, in the absence of these risk factors, such tumors can be treated by local excision, by endoscopic resection or transanal resection. Therefore, further examination of the relationship between allelic status and lymph node status is warranted in future studies.

The DNA of tumor tissues is inevitably not homogeneous because of stromal cell contamination or the genetic heterogeneity of tumor cell populations, which have also been proposed to cause a wide range of allelic losses. In conventional RFLP (restriction fragment length polymorphism) or PCR-based RFLP analysis, to detect allelic loss the proportion of tumor cells in the sample must exceed at least 50% of the total cells, and a large amount of DNA is required. Clinical samples are often contaminated with normal cells, and the tumor cellularity is sometimes less than 50%. In such cases, conventional techniques cannot detect allelic loss and the allelic status is considered to be retained. This suggests that conventional techniques cannot be used to detect clear associations between allelic loss and prognosis. Here, blunt-end SSCP analysis, which can detect allelic losses when the tumor cellularity of the sample is around 10% and requires only a small amount of DNA, was used. These advantages enabled the detection of allelic losses using small amounts of DNA obtained from biopsy specimens, surgical materials and formalin-fixed, paraffin-embedded sections. The method is clinically very useful, because surgical materials and biopsy samples of cancer are usually contaminated with many normal cells.

It was found that the number of allelic losses was not associated with the prognosis of CRC. However, Choi *et al.* showed that the number of allelic losses was associated with prognosis, this factor still being significant in multivariate analysis (8). Because they had examined eight chromosomes (3p, 4p, 5q, 8p, 9p, 13q, 17p and 18q), this conflicting result might be explained by the difference in the chromosomes examined. If the level of chromosomal loss is an important

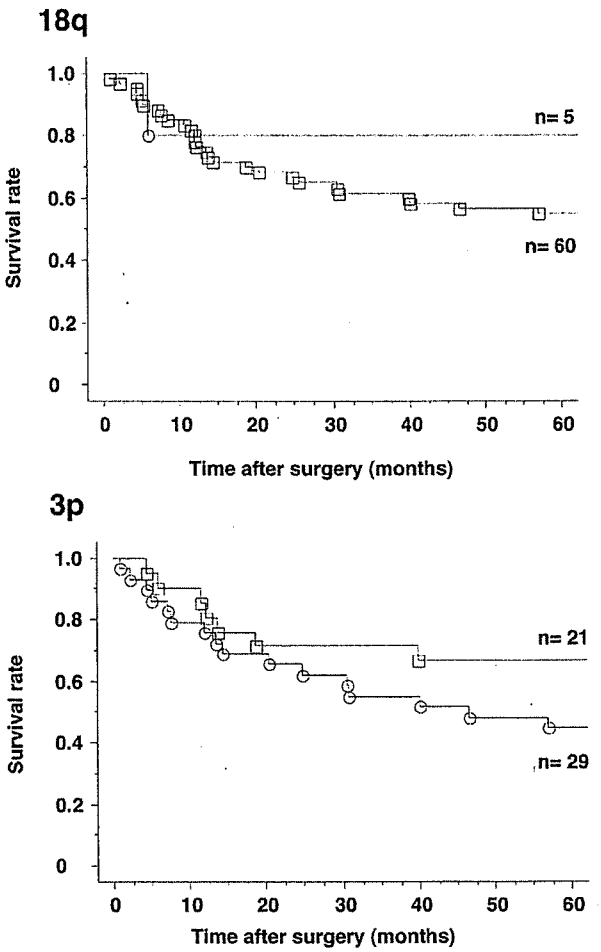

Figure 2. *Disease-free survival curves in stage III CRC. Patients with allelic loss at 18q showed worse survival than those without allelic loss at 18q, and patients with allelic loss at 3p showed better survival than those without this allelic loss. These differences were not significant.* □: *Lost,* ○: *Retained.*

prognostic factor, then it is of importance to determine which chromosomes are important for prognosis and how many chromosomes are to be examined. On the other hand, Rooney *et al.* obtained contrary results using comparative genomic hybridization (21). In their study, Dukes' C patients with more than two genomic aberrations had a better survival rate than did patients with fewer regions. Rooney *et al.* also showed that single genomic instabilities were not correlated with survival.

The allelic loss of chromosome 17p is a very common event in CRC. Although the allelic status of chromosome 17p is correlated with some clinicopathological backgrounds, only a small number of reports have suggested the prognostic importance of this allelic loss (1, 8), while other reports, including this study, showed no correlation between prognosis and allelic loss (15, 17-19). For p53, intragenic polymorphic markers were used. Even where the intragenic markers were informative, there was no correlation between prognosis and allelic loss of p53 (data not shown).

The allelic loss of chromosome 22q is relatively frequent in CRC (22, 23, 25, 31). However, there is no report of a tumor suppressor gene on 22q. Although Iino *et al.* have shown that allelic loss of chromosome 22q was correlated with lymph node metastasis (31), there have been no reports of a relationship between the allelic loss of chromosome 22q and prognosis. No relationship was found between the allelic loss of 22q and the clinicopathological background or prognosis, meaning that it probably is not a prognostic factor in CRC patients.

The allelic loss of chromosome 3p is also relatively frequent in CRC, and detailed deletion mapping studies have suggested the existence of tumor suppressor genes on this chromosome, although non have been reported. Iniesta *et al.* showed that allelic loss of 3p was significantly associated with worse prognosis in CRC patients (4). Although theirs was the first report to demonstrate the prognostic significance of the allelic loss of 3p, our study revealed no relationship between the clinicopathological background and allelic status. Choi *et al.* suggested that allelic loss of 3p was correlated with cancer-related death (8). Blaker *et al.* (25) showed preferential loss of chromosome 3p in CRC. However, no additional studies have supported this result, and we were unable to show a relationship between the clinicopathological background or prognosis and allelic loss of 3p.

In summary, although allelic status was not associated with prognosis in CRC patients without adjuvant chemotherapy, it was significantly associated with lymph node metastasis, and a combination of the allelic status and lymphatic invasion status can be used to predict the lymph node status before radical surgery. When allelic loss and lymphatic invasion are not detected after local excision, additional lymph node resection is not required.

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