別刷

# 癌と化学療法

VOL.34(2007)

癌と化学療法社

# 大腸癌腹膜播種再発に伴う腸閉塞に対する切除術の検討

加藤 健志 三宅 泰裕 土井 貴司 大島 一輝 半田 理雄
星 美奈子 間狩 洋一 大島 聡 飯島 正平 黒川 英司
吉川 宣輝*

〔*Jpn J Cancer Chemother* 34(12): 1949-1951, *November*, 2007〕

A Clinical Significance of Peritoneal Carcinomatosis to Recurrent Colorectal Cancer with Ileus: Takeshi Kato, Yasuhiro Miyake, Takashi Doi, Kazuteru Oshima, Rio Handa, Minako Hoshi, Yoichi Makari, Satoshi Oshima, Shouhei Iijima, Eiji Kurokawa and Nobuteru Kikkawa (*Dept. of Surgery, Minoh City Hospital Gastrointestinal Research Center*)

*Summary*

We studied a clinical significance of peritoneal carcinomatosis to metastatic recurrent colorectal cancer with ileus. The subjects were 16 patients with ileus confirmed in 1995-2005. Of the 16 patients, 7 had surgical treatment and the other 9 had conservative treatment. The median survival time of the 16 patients was 98 days; the 7 patients of surgical treatment were 235 days; the 9 patients of conservative treatment were only 67 days. Only 4 patients survived more than 200 days. These 4 patients had surgical treatment, and were the cases where no peritoneal fluid was confirmed and the metastatic tumor was excised. However, only 2 patients improved in QOL. The 3 patients in the surgical treatment were aggravated. An optimum resection may prolong a survival time and patient's QOL could be improved. On the other hand, an extended resection may contribute to shorting a survival time. **Key words**: Colorectal cancer, Peritoneal carcinomatosis, Ileus

**要旨** 大腸癌の腹膜播種再発に対しては有効な治療法がなく，腸閉塞を合併するとQOLの低下が著しく治療に難渋することが多い。そこで症状を軽減する緩和療法を目的として，手術療法が選択されるが無理な手術療法は症状を悪化する可能性がある。大腸癌の腹膜播種が原因で，腸閉塞を併発した症例は1995年から2005年までに16例認め，そのうち手術を施行した症例は7例で，9例は保存的に治療していた。生存期間の中央値は全16例で98日，手術を施行した症例で235日，保存的治療を施行した症例は67日であった。術後200日以上生存した症例が4例で，術前検査で腹水を認めず播種巣をすべて切除した症例であった。術後のQOLが改善した症例は2例で，3例はQOLが明らかに悪化した。術前に腹水を認めた症例やバイパス術や小腸瘻を造設した症例は，QOLや予後を悪化する可能性があり，手術適応には慎重でなければならない。

## はじめに

大腸癌の腹膜播種再発の予後は不良で，しかも腸閉塞を合併することが多く，QOLを著しく低下させる。症状軽快を目的としてイレウス管や胃管を挿入するが，症状が軽快しないことや挿入と抜去を繰り返すことが多く，手術療法が考慮される。手術療法はQOLや予後を改善する可能性はあるが，手術適応を誤るとQOLに悪影響を及ぼすばかりでなく，生存期間を短縮する。

今回は，当院で腹膜播種が原因で腸閉塞を併発した症例について検討したので報告する。

## I. 対象，方法

1995年から2005年までに，当センターで大腸切除術を施行した大腸癌1,093例のうち，術後に腸閉塞を併発した症例は59例であった。59例中43例は癒着が原因の腸閉塞で，37例は保存的に軽快し，6例に対して手術を施行した。腹膜播種が原因で腸閉塞を合併した症例は16例で，手術を施行した症例は7例であった。今回はこの16例，特に手術を施行した7例について検討した。

## II. 結 果

### 1. 保存的治療選択の理由

16例中9例に保存的治療を選択し，その理由を表1に示した。術前検査にて多量の腹水を認め，開腹術が困難だと診断した症例が7例，イレウス管造影検査や腹部CT検査にて狭窄部位を複数認め，手術困難と診断した症例が3例，全身状態が悪く手術が不可能と判断した症例が

連絡先: 〒562-8562 箕面市萱野5-7-1 *箕面市立病院・胃腸センター・外科
加藤 健志

0385-0684/07/¥500/論文/JCLS

表 1 保存的治療を選択した9例の理由

| | |
|---|---|
| ・腹水貯留 | 7例 |
| ・転移巣多数 | 4例 |
| ・全身状態不良 | 3例 |

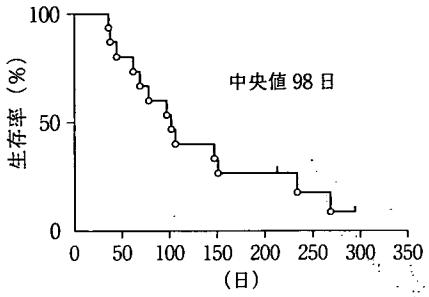



図 1 腹膜播種再発で腸閉塞と診断した16例の生存率

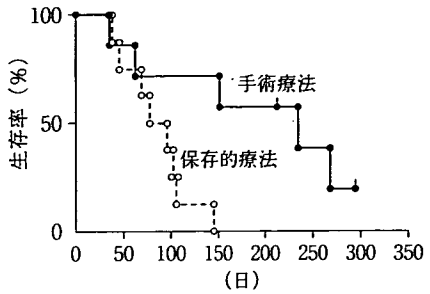



図 2 治療法別の生存率

表 2 全手術症例（7例）

| 症例 | 性別 | 手術時年齢 | PS | CTで腹水 | PET FDG取り込み | 術式 播種巣 | 術式 再建 | 播種の数（箇所） | 術中腹水 | 他臓器転移 | 術後QOL | 術後化学療法 | 術後生存期間（日） | 生死 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 女性 | 87 | 1 | なし | あり | sR0 | 小腸切除 | 3 | なし | リンパ | 改善 | なし | 213 | 生存中 |
| 2 | 男性 | 72 | 2 | なし | あり | sR0 | 小腸切除 | 3 | なし | 肝 | 改善 | FOLFOX | 294 | 生存中 |
| 3 | 女性 | 73 | 2 | あり | 施行せず | 非切除 | バイパス | 11 | 少量 | 肝 | 低下 | なし | 62 | 在院死亡 |
| 4 | 女性 | 58 | 1 | なし | 施行せず | sR2 | 小腸瘻 | 8 | 少量 | リンパ | 変化なし | FU/LV | 268 | 死亡 |
| 5 | 男性 | 63 | 1 | なし | 施行せず | sR2 | 小腸切除 | 5 | 少量 | 肝 | 変化なし | なし | 234 | 死亡 |
| 6 | 男性 | 73 | 2 | あり | 施行せず | 非切除 | 小腸瘻 | 多数 | 中等量 | 肝 | 低下 | なし | 36 | 在院死亡 |
| 7 | 女性 | 55 | 2 | あり | 施行せず | 非切除 | 小腸瘻 | 多数 | 少量 | 肝 | 低下 | なし | 151 | 在院死亡 |

3例であった。

2. 生 存 率

腹膜播種再発で腸閉塞と診断した16例の生存曲線を図1に示した。生存期間の中央値はわずか98日であった。図2に手術療法と保存療法の生存曲線を示したが，保存療法では生存期間の中央値が67日であり，手術療法では235日であった。

3. 手 術 症 例

表2に7例の手術症例を示した。女性が4例で，平均年齢は69歳であった。術前の全身状態をperformance status（ECOG）で表すと，1または2で全身状態が悪い症例が多かった。術前検査で腹水を認めた症例が3例，FDG-PETを2例に施行し，2例ともに転移巣を確認することが可能であった。手術術式は切除術が4例で，2例は肉眼的には完全に播種巣を切除することができたsR0で，3例は非切除であった。再建術式は，小腸吻合術3例，バイパス術1例，小腸瘻造設術が3例であった。術中腹水を認めた症例は5例で，腹膜播種巣が3か所であった症例が2例で，その他の症例は5か所以上腹膜播種巣を認めた。他臓器転移は，肝転移5例，リンパ節転移が2例で全例に他臓器転移を認めた。術後QOLが明らかに改善した症例は2例，不変の2例は術後食物摂取が可能となったが，短腸症候群のため吸収障害を認め，十分食事ができなかった。残りの3例は症状の改善は認めず，QOLは明らかに低下した。術後200日以上生存した症例が4例，残りの3例は在院死亡であった。

4. 術後200日以上生存症例

術後200日以上生存した症例の特徴を表3にまとめた。術前検査所見では腹水を認めず，腹膜播種巣をFDG-PETで指摘でき，その播種巣を完全に切除することが可能であると診断した症例であった。また術中所見では播種巣を完全に切除し，腹水を少量認めるのみの症例で

表 3 術後200日以上生存した症例

- 術前CTにて腹水を認めない
- 術前PET-CTにて病巣の指摘が可能
- 術中播種巣の切除が可能
- 術中腹水が少量

あった。一方QOLが低下した症例は，術前CTで腹水を認めた症例で，播種巣を切除することが困難な症例であった。

## III. 考 察

大腸癌における腹膜播種は予後不良ではあるが，原発症例であれば外科的切除により長期生存も期待できる[1-3]。北条[4]はH0，P1，P2症例で，岡ら[5]は肝転移に関係なく腹膜播種病巣の切除により延命効果があると報告している。また，山口ら[6]は腹膜播種症例の予後因子を多変量解析し，腫瘍の分化度，肝転移，リンパ節転移の程度であるとし，リンパ節郭清をD>nとし，P1H0，P2H0，P1H1までの症例では，肝切除を同時に行うと長期予後が期待できると報告している。しかしこれらの報告は原発症例に対する治療成績で，再発症例に対する治療成績の報告は少なく，さらに腸閉塞症例に対する報告は少ない。

今回の検討では，腹膜播種再発が原因で腸閉塞を伴った症例の予後は不良で，その中間値は98日，特に手術を行わなかった症例の生存期間の生存値はわずか67日であった。またこれらの症例は，イレウス管や胃管の挿入を繰り返すことが多くQOLもたいへん悪いため，緩和医療の対象となることが多い。今回の検討では，緩和的な手術療法を行った7例中4例で200日以上の長期生存を得ることができ，QOLが改善した症例を2例認めた。しかし3例が在院死亡し，術後のQOLは著しく低下した。手術療法は，腹膜播種再発が原因で腸閉塞を伴った症例に対しては有効な治療法ではあるが，その適応を誤るとQOLを悪化させる可能性があるので手術適応には慎重でなければならない。その適応は術前検査で腹水がなく，PET検査やCT検査にて播種巣が指摘できた症例で，全身状態のよい症例である。逆に，多量の腹水を認める症例に対する手術療法には慎重でなければならない。

本論文の要旨は第29回日本癌局所療法研究会において発表した。

## 文 献

1) 栗野友太，奥山和明，小出義雄・他: 腹膜播種性大腸癌の臨床病理学的検討．日本大腸肛門病会誌 **46**: 756-760, 1993.
2) 山村卓也，花井 彰，及川 博・他: 大腸癌非治癒手術因子の検討．日消外会誌 **24**: 2536-2541, 1991.
3) 友田博次，竹内博昭，武富紹信・他: 腹膜播種を伴った大腸癌の予後．外科 **60**: 301-305, 1998.
4) 北条慶一: 大腸癌高度伸展例（Stage IV，V）の拡大手術と成績．日外会誌 **90**: 1428-1431, 1989.
5) 岡 正朗，飯塚徳男，鈴木 敞・他: 腹膜播種を伴った大腸癌症例の臨床病理学的検討．日臨外会誌 **54**(10): 2535-2539, 1993.
6) 山口由美，柴田俊輔，石黒 稔・他: 腹膜播種を伴う大腸癌切除例の臨床病理学的検討．臨床外科 **59**(2): 181-186, 2004.

別刷

# 癌と化学療法

VOL.34(2007)

癌と化学療法社

# 鼠径ヘルニア嚢転移を来した上行結腸癌の1例

三宅 泰裕　加藤 健志　片山 欽三　土井 貴司　大島 一輝
半田 理雄　星 美奈子　間狩 洋一　大島 聡　飯島 正平
黒川 英司　吉川 宣輝*

〔*Jpn J Cancer Chemother* 34(12): 2016-2018, *November*, 2007〕

A Case of Ascending Colon Carcinoma Metastasized to an Inguinal Hernia Sac: Yasuhiro Miyake, Takeshi Kato, Kinzo Katayama, Takashi Doi, Kazuteru Oshima, Rio Handa, Minako Hoshi, Yoichi Makari, Satoshi Oshima, Shohei Iijima, Eiji Kurokawa and Nobuteru Kikkawa (*Dept. of Surgery, Minoh City Hospital Gastrointestinal Research Center*)

*Summary*

While inguinal hernia is one of the most common diseases, metastatic cancer of an inguinal hernia sac is rare. We report a case of ascending colon cancer metastasized to an inguinal hernia sac. A 60-year-old man, who was undergone a right hemicolectomy for an ascending colon cancer, was pointed out a palpable inguinal mass at one year and eight months after the operation. He was diagnosed as inguinal hernia, and herniorrhaphy was performed. In the operation, a tumor of the inguinal hernia sac, which invaded to spermatic cord, could be found and was removed with right testis. Bassini's method was performed after the resection of the inguinal tumor. Histological examination revealed that the tumor was metastasis of colon carcinoma. Examination of the entire body showed no other metastasis. As for the advanced colon cancer, we need to mention the possibility of metastatic saccular tumor. **Key words**: Colon carcinoma, Inguinal hernia, Metastatic saccular tumor

**要旨**　鼠径ヘルニアおよび悪性腫瘍はそれぞれが高頻度でみられるにもかかわらず，鼠径ヘルニア嚢に悪性腫瘍が存在する頻度は少ない。今回われわれは，上行結腸癌が右鼠径ヘルニアに転移した症例を経験したので報告する。症例は60歳台前半の男性。上行結腸癌術後1年8か月を経過し，右鼠径部の腫脹を認めた。右鼠径ヘルニアの診断にて手術を施行した。ヘルニア先端に約3 cm大の腫瘤が精索に浸潤していたため，悪性疾患を疑い，右睾丸とともに精索と腫瘤を切除し，Bassini法にて補強した。上行結腸癌と同様の腺癌を認め，上行結腸癌の鼠径ヘルニア嚢転移と診断した。その後の全身精査では他に転移巣を認めず，単発性鼠径ヘルニア転移と考えられた。進行大腸癌においては鼠径ヘルニア転移が生じることを念頭に置き，診療に当たるべきであると考えられた。

## はじめに

鼠径ヘルニア嚢に腫瘍が存在する鼠径ヘルニア嚢腫瘍はまれな疾患であり，そのなかで転移性腫瘍を認めるものは，転移性鼠径ヘルニア嚢腫瘍と称されている。その原発部位は結腸癌である可能性が高いが，その報告は多くない。今回われわれは，上行結腸癌術後1年8か月を経過し，転移性鼠径ヘルニア嚢腫瘍として再発した症例を経験したので報告する。

## I. 症　例

**患者**: 60歳台前半，男性。

**主訴**: 右鼠径部腫脹。

**家族歴**: 特記すべきものなし。

**既往歴**: 特記すべきものなし。

**現病歴**: 2004年7月上行結腸癌のため，右半結腸切除術を施行し（中分化型腺癌，pSS，ly0，v0，pN1，P0，H0，Stage IIIa），術後半年間の補助化学療法として5-FU/LVが施行された。2006年4月ごろより右鼠径部の腫瘤を認めたが，腹部CT検査では肝臓や肺野には再発所見を認めず，腹水も認めなかったため，右側鼠径ヘルニアと診断された。

**現症**: 身長165.0 cm，体重62.0 kg，血圧120/80 mmHg，脈拍86/分，体温36.5℃，眼瞼結膜に貧血なく，眼球結膜に黄疸はなかった。心肺に特記すべき所見はなく，腹部は平坦・軟であり腹部正中に手術創を認め

連絡先: 〒562-8562 箕面市萱野5-7-1　* 箕面市立病院・胃腸センター・外科
三宅 泰裕

0385-0684/07/¥500/論文/JCLS

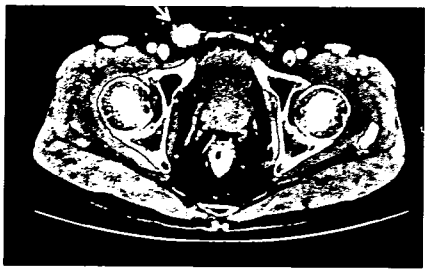


図1 腹部造影CT検査
右鼠径部に大きさ約3cmの腫瘤（矢印）を認め，周囲への炎症所見を認めた。

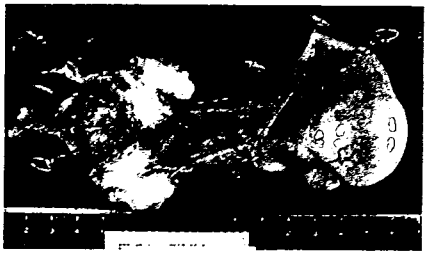


図2 摘出標本
精索に浸潤した3cm大の充実性腫瘤を認め，精巣とともに切除した。

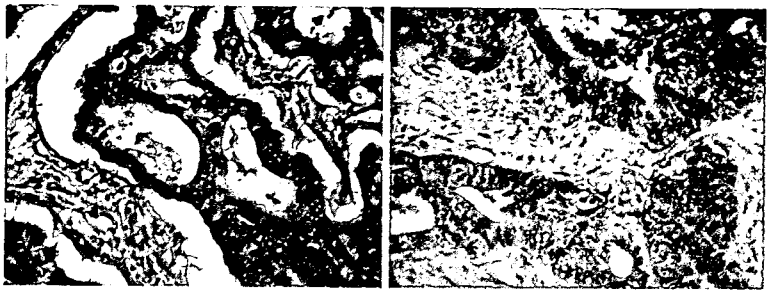


図3 病理組織検査（HE染色）
左側は上行結腸腫瘍，右側は鼠径ヘルニア嚢腫瘍。同様の中分化型腺癌で上行結腸癌の鼠径ヘルニア嚢転移と診断された。

た。右鼠径部に大きさ約3cmの腫大を認めた。同部に発赤なく圧痛は認めなかった。

**入院時検査成績**: WBC 8,300/μL，RBC 466×10⁴/μL，Hb 15.7 g/dL，Hct 45.4%，Plt 25.8×10⁴/μL，生化学検査ではAST 52 U/L，ALT 56 U/L，ALP 348 U/Lと軽度高値を示した。腫瘍マーカーはCEA 1.9 ng/dL（正常値<5.0 ng/dL），CA19-9 3.0 U/mL（正常値<37.0 U/mL）と正常範囲であった。

**腹部造影CT検査**: 肺野に異常所見なく，肝転移認めず，腹水も認めなかった。右鼠径部に大きさ約3cmの腫瘤を認め，周囲への炎症所見を認めた（図1）。

以上より右鼠径ヘルニア腫瘍と診断し，2006年5月手術を施行した。

**手術所見**: 右鼠径部を皮膚切開し，外ヘルニア嚢を認め，その先端で約3cm大の腫瘤があり，精索に強固に浸潤していた。精索と腫瘤の剝離は困難であると判断し，右睾丸とともに右精索と腫瘤を一塊に切除した。Bassini法にて補強を行った。

**切除標本**: 精索に浸潤した3cm大の充実性腫瘤を認めた（図2）。

**病理組織検査**: ヘルニア嚢の先端から精索に浸潤増殖を示す中分化型腺癌を認めた。前回切除した上行結腸癌と組織型が一致することから，上行結腸癌の鼠径ヘルニア嚢転移と診断した（図3）。転移形式としては血行性転移やリンパ行性転移の可能性が低いことから，腹膜播種により鼠径ヘルニア嚢に転移の可能性が高いと考えられた。現在，患者は外来化学療法を継続中である。

## II. 考　察

鼠径ヘルニア手術は，外科手術のなかで最も一般的な手術であり広く行われているが，鼠径ヘルニア嚢内に悪性腫瘍が発見されることは比較的まれとされている。Yoellらは鼠径ヘルニア手術を施行したうち，0.4%に悪性腫瘍が発見されたと報告し[1]，Nicholsonらは0.07%に転移性ヘルニア腫瘍が発見されたと報告している[2]。鼠径ヘルニア腫瘍は，ヘルニア嚢と腫瘍の位置関係によって，① ヘルニア嚢内腫瘍（intrasaccular tumors），② ヘルニア嚢腫瘍（saccular tumors），③ ヘルニア嚢外腫瘍（extrasaccular tumors）3種類に分類される[2]。① はヘルニア嚢内に嵌頓する腫瘍によるもので膀胱癌，虫垂癌，大網転移性病変が含まれ，② はヘルニア嚢そのものを含む腫瘍であり，悪性腫瘍の腹膜転移が含まれ，③ は腫瘍がヘルニア嚢の外部に存在しヘルニアのように突出する腫瘍であり，脂肪腫，脂肪肉腫やリンパ節転移などが含まれている。今回，われわれの経験した症例はヘルニア嚢の先端そのものが腫瘍であり，精索に浸潤していたため，② ヘルニア嚢腫瘍と診断した。

Matsumotoらは，鼠径ヘルニア嚢内に大腸癌が存在したと報告のあった25症例を集計したが，そのうち21例に主病変がヘルニア嚢内に存在したヘルニア嚢内腫瘍と

しており，転移性ヘルニア嚢腫瘍であったものはわずか4例であった[3]。また，ヘルニア嚢内に大腸癌からの腫瘍が存在したと報告したものの多くは中高年の男性であり，わずかに1例のみ横田らが，女性での大腸癌の鼠径ヘルニア嚢転移を報告している[4]。大腸癌の鼠径ヘルニア嚢転移の頻度は高くはないが，結腸癌病歴のある症例の鼠径ヘルニアでは肉眼的にヘルニア嚢が正常ではないと考えられた場合には病理学的検索が必要であると報告されている[5,6]。鼠径ヘルニア嚢転移の病態として腹膜播種と考えられ，一般的には予後不良とされる。また，転移性鼠径ヘルニア嚢腫瘍の報告例には多発する腹膜播腫を認める症例が多いが，まれに他に腹膜播腫を認めない単発性鼠径ヘルニア嚢転移の報告も認められる[4]。自験例においても術後PET/CTなどの全身検索を行ったが，他に転移巣は認められず単発性鼠径ヘルニア嚢転移と考えられた。転移形式として大腸癌の腹膜播腫が考えられたため，術後は当科で進行再発大腸癌に行っている多剤併用化学療法を施行し，無再発生存中である。

## 結　語

進行大腸癌においては転移性鼠径ヘルニア嚢腫瘍が生じることを念頭に置き，検査・処置を行うべきであると考えられた。

本論文の要旨は第29回日本癌局所療法研究会において発表した。

## 文　献

1) Yoell JH: Surprises in hernial sacs; diagnosis of tumors by microscopic examination. *Calf Med* **91**: 146-148, 1959.
2) Nicholson CP, Donohue JH, Thompson GB, *et al*: A study of metastatic cancer found during inguinal hernia repair. *Cancer* **69**: 3008-3011, 1992.
3) Matsumoto G, Ise H, Inoue H, *et al*: Metastatic colon carcinoma found within an inguinal hernia sac: report of a case. *Surg Today* **30**: 74-77, 2000.
4) 横田良一，秦　温信，松岡伸一・他: S状結腸癌の鼠径ヘルニア嚢転移の1例．日臨外会誌 **64**(4): 999-1002, 2003.
5) 志田　大，吉見富洋，小形幸代・他: 胆管細胞癌の転移性鼠径ヘルニア嚢腫瘍の1例．日消外会誌 **33**(11): 1816-1820, 2000.
6) 熊澤博久，高木眞人，岩畔　彪・他: ヘルニア嚢の組織所見から腹部消化器癌を検索しえた2症例．外科診療 **6**: 883-886, 1991.

J Hum Genet (2007) 52:804–819
DOI 10.1007/s10038-007-0186-6

ORIGINAL ARTICLE

# Genetic variations and haplotype structures of the *DPYD* gene encoding dihydropyrimidine dehydrogenase in Japanese and their ethnic differences

**Keiko Maekawa · Mayumi Saeki · Yoshiro Saito · Shogo Ozawa · Kouichi Kurose · Nahoko Kaniwa · Manabu Kawamoto · Naoyuki Kamatani · Ken Kato · Tetsuya Hamaguchi · Yasuhide Yamada · Kuniaki Shirao · Yasuhiro Shimada · Manabu Muto · Toshihiko Doi · Atsushi Ohtsu · Teruhiko Yoshida · Yasuhiro Matsumura · Nagahiro Saijo · Jun-ichi Sawada**

Received: 30 May 2007 / Accepted: 26 July 2007 / Published online: 9 September 2007


**Abstract** Dihydropyrimidine dehydrogenase (DPD) is an inactivating and rate-limiting enzyme for 5-fluorouracil (5-FU), and its deficiency is associated with a risk for developing a severe or fatal toxicity to 5-FU. In this study, to search for genetic variations of *DPYD* encoding DPD in Japanese, the putative promoter region, all exons, and flanking introns of *DPYD* were sequenced from 341 subjects including cancer patients treated with 5-FU. Fifty-five genetic variations, including 38 novel ones, were found and consisted of 4 in the 5′-flanking region, 21 (5 synonymous and 16 nonsynonymous) in the coding exons, and 30 in the introns. Nine novel nonsynonymous SNPs, 29C>A (Ala10Glu), 325T>A (Tyr109Asn), 451A>G (Asn151Asp), 733A>T (Ile245Phe), 793G>A (Glu265Lys), 1543G>A (Val515Ile), 1572T>G (Phe524Leu), 1666A>C (Ser556-Arg), and 2678A>G (Asn893Ser), were found at allele frequencies between 0.15 and 0.88%. Two known nonsynonymous variations reported only in Japanese, 1003G>T (**11*, Val335Leu) and 2303C>A (Thr768Lys), were found at allele frequencies of 0.15 and 2.8%, respectively. SNP and haplotype distributions in Japanese were quite different from those reported previously in Caucasians. This study provides fundamental information for pharmacogenetic studies for evaluating the efficacy and toxicity of 5-FU in Japanese and probably East Asians.

**Keywords** *DPYD* · SNP · Haplotype · Japanese · 5-fluorouracil

K. Maekawa (✉) · Y. Saito · J. Sawada
Division of Biochemistry and Immunochemistry,
National Institute of Health Sciences,
1-18-1 Kamiyoga, Setagaya-ku,
Tokyo 158-8501, Japan
e-mail: maekawa@nihs.go.jp

K. Maekawa · M. Saeki · Y. Saito · S. Ozawa · K. Kurose · N. Kaniwa · J. Sawada
Project Team for Pharmacogenetics,
National Institute of Health Sciences, Tokyo, Japan

S. Ozawa
Division of Pharmacology,
National Institute of Health Sciences, Tokyo, Japan

K. Kurose · N. Kaniwa
Division of Medicinal Safety Science,
National Institute of Health Sciences, Tokyo, Japan

M. Kawamoto · N. Kamatani
Division of Genomic Medicine,
Department of Advanced Biomedical Engineering and Science,
Tokyo Women's Medical University, Tokyo, Japan

K. Kato · T. Hamaguchi · Y. Yamada · K. Shirao · Y. Shimada
Gastrointestinal Oncology Division, National Cancer Center Hospital, National Cancer Center, Tokyo, Japan

M. Muto
Gastrointestinal Oncology Division,
National Cancer Center Hospital East, Kashiwa, Japan

T. Doi · A. Ohtsu
Division of GI Oncology/Digestive Endoscopy,
National Cancer Center Hospital East, Kashiwa, Japan

T. Yoshida
Genetics Division, National Cancer Center Research Institute,
National Cancer Center, Tokyo, Japan

Y. Matsumura
Research Center of Innovative Oncology,
National Cancer Center Hospital East, Kashiwa, Japan

N. Saijo
Deputy Director, National Cancer Center Hospital East, Kashiwa, Japan

## Introduction

Dihydropyrimidine dehydrogenase (DPD) is an inactivating and rate-limiting enzyme for 5-fluorouracil (5-FU), which is used in various therapeutic regimens for gastrointestinal, breast and head/neck cancers (Grem 1996). While the antitumor effect of 5-FU is exerted via anabolic pathways responsible for its intracellular conversion into anti-proliferative nucleotides, DPD affects 5-FU availability by rapidly degrading it to 5, 6-dihydrofluorouracil (DHFU) (Heggie et al. 1987). The importance of DPD in 5-FU metabolism was also highlighted by a lethal drug interaction between 5-FU and the antiviral agent sorivudine. Due to inhibition of DPD by a sorivudine metabolite, severe systemic exposure to 5-FU caused several acute deaths in Japan (Nishiyama et al. 2000).

5-FU catabolism occurs in various tissues, including tumors, but is highest in the liver (Naguib et al. 1985; Lu et al. 1993). Wide variations in DPD activity (8- to 21-fold) were shown in Caucasians, and 3–5% of Caucasians had reduced DPD activity (Etienne et al. 1994; Lu et al. 1998). This variability, which is partially attributed to genetic defects of the DPD gene (*DPYD*), leads to differential responses of cancer patients, resistance to or increased toxicity of 5-FU (van Kuilenburg 2004). Complete DPD deficiency is also associated with the inherited metabolic disorder, thymine-uraciluria, which is characterized by neurological problems in pediatric patients (Bakkeren et al. 1984).

To date, at least 30 variant *DPYD* alleles have been published, with or without deleterious impact upon DPD activity (Gross et al. 2003; Ogura et al. 2005; Seck et al. 2005; van Kuilenburg 2004; Zhu et al. 2004). Of these variations, a splice site polymorphism, IVS14 + 1G>A, which causes skipping of exon 14, is occasionally detected in North Europeans with allele frequencies of 0.01–0.02 (van Kuilenburg 2004). Detection of IVS14 + 1G>A in patients suffering from 5-FU-associated grade 3 or 4 toxicity revealed that 24–28% of them were heterozygous or homozygous for this single nucleotide polymorphism (SNP) (van Kuilenburg 2004). However, this SNP has not been reported in Japanese and African-Americans. Recently, Ogura et al. (2005) have shown that a Japanese population exhibits a large degree of interindividual variations in DPD activity of peripheral blood mononuclear cells. They also identified a novel variation, 1097G>C (Gly366Ala), in a healthy volunteer with the lowest DPD activity and demonstrated that the 366Ala variant has reduced activity towards 5-FU in vitro. At present, however, information on variant alleles with clinical relevance in Japanese is limited and cannot fully explain polymorphic DPD activity.

In this study, we searched for genetic variations in *DPYD* by sequencing 5′ regulatory regions, all exons and surrounding introns from 341 Japanese subjects. Fifty-five variations including nine novel nonsynonymous ones were identified. Then, linkage disequilibrium (LD) and haplotype analyses were performed to clarify the *DPYD* haplotype structures in Japanese.

## Materials and methods

### Human DNA samples

Three hundred and forty-one Japanese subjects in this study included 263 cancer patients and 78 healthy volunteers. All 263 patients were administered 5-FU or tegafur for treatment of various cancers (mainly stomach and colon) at the National Cancer Center, and blood samples were collected prior to the fluoropyrimidine chemotherapy. The healthy volunteers were recruited at the Tokyo Women's Medical University. DNA was extracted from the blood of cancer patients and Epstein-Barr virus-transformed lymphoblastoid cells derived from healthy volunteers. Written informed consent was obtained from all participating subjects. The ethical review boards of the National Cancer Center, the Tokyo Women's Medical University and the National Institute of Health Sciences approved this study.

### PCR conditions for DNA sequencing

To amplify 22 exons (exons 2–23) of *DPYD*, multiplex PCRs were performed by using four sets of mixed primers (mix 1 to mix 4 of "first PCR" in Table 1). Namely, five exonic fragments were simultaneously amplified from 50 ng of genomic DNA using 0.625 units of Ex-Taq (Takara Bio. Inc., Shiga, Japan) with 0.20 μM primers. Because of the high GC content in exon 1 of *DPYD*, this region was separately amplified from 50 ng of genomic DNA with 2.5 units of LA-Taq and 0.2 μM primers (listed in Table 1) in GC buffer I (Takara Bio. Inc.). The first PCR conditions were 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 58°C for 1 min, and 72°C for 2 min; and then a final extension for 7 min at 72°C. Next, each exon was amplified separately from the first PCR products by nested PCR (2nd PCR) using the primer sets (0.2 μM) listed in "second PCR" of Table 1. The second PCR conditions were the same as those of the first PCR, and LA-Taq (2.5 units) for exon 1 and Ex-Taq (0.625 units) for exons 2–23 were used. All PCR primers were designed in the flanking intronic sites to analyze the exon-intron splice junctions. The PCR products were treated with a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH) and sequenced directly on both strands using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems,

**Table 1** Primer sequences for human *DPYD*

| Amplified and sequenced region | | | Forward primer | | Reverse primer | | PCR product (bp) |
|---|---|---|---|---|---|---|---|
| | | | Sequences (5' to 3') | Position[a] | Sequences (5' to 3') | Position[a] | |
| First PCR | 5'-UTR to exon 1 | | GTTCTGGAAGGTAATCTGATGG | 52207178 | ACGACATACAGGAGGTGAAG | 52205443 | 1,736 |
| | Mix 1 | Exon 2 | CTACTTGGGAGACTAAGGTG | 52168526 | GTATCATTGTGTCATTAGGC | 52167832 | 695 |
| | | Exon 3 | TCCCTTCATCTTAGTCAATG | 52113605 | CTGAGGCTTAACATTTATGC | 52112876 | 730 |
| | | Exon 4 | TCTGAGAGGAGGGACAGTTA | 52025660 | AATCACAACTTGGAAGTGCT | 52025165 | 496 |
| | | Exon 5 | AAATGGAGGATAACCTGAGT | 52007046 | TAATAACCTGCTGGGATTGC | 52006234 | 813 |
| | | Exon 6 | AGAGGAGAGGCACTTAATGT | 51984772 | TGCTTCAAGCCAACTGCAAA | 51984115 | 658 |
| | Mix 2 | Exon 7 | CTCAAATAATAGTGCCATAGG | 51977410 | CAGTAGACAGACAAATGCCC | 51976498 | 913 |
| | | Exon 8 | CACATCGTGCTTTGAACATA | 51964415 | CCAACTCCATCCTTTATGAT | 51963667 | 749 |
| | | Exons 9 and 10 | TGAGGCAAGAATATAACCTG | 51880431 | TCCGTATGTGTCTTATTACC | 51877795 | 2,637 |
| | | Exon 11 | AGAAATACCTTATGATGCCG | 51859160 | GCCTTTTGAATCAAGATTGC | 51858562 | 599 |
| | | Exon 16 | CTCCCTATGCTTCAGTTCAC | 51658925 | TGCCGTGCCCCATTTACTAC | 51658114 | 812 |
| | Mix 3 | Exon 12 | CCGCTCTGAAACATTGACCA | 51834944 | CTGGGATTATAGGCATTAGG | 51834279 | 666 |
| | | Exon 13 | GCCCATATCTCTGAGCACTA | 51801258 | ATCTTTGTTGCTTCCTAGAC | 51800450 | 809 |
| | | Exon 14 | CCTTCACTGATTTACATCGG | 51735640 | CCAGCCACATACAGTGAAAA | 51734704 | 937 |
| | | Exon 15 | AGCCAGTAAAATCCTCTCTA | 51667711 | TATGGAAAACCTGCTGACTA | 51666815 | 897 |
| | | Exon 23 | TGGAAAGACCCGAACTCTGC | 51364409 | AGCGAAGGGGATTTTACTTA | 51363336 | 1,074 |
| | Mix 4 | Exons 17 and 18 | TTCTAAAGGCTCTGTTGAGG | 51591491 | TGGCAAAAGAACTGAGAGAC | 51589933 | 1,559 |
| | | Exon 19 | CGTGGATTCAAGCAGTTTTC | 51520500 | AGACAGTGGGTTCGTAAGCC | 51519586 | 915 |
| | | Exon 20 | CTGTGACACCATTACCATTG | 51478435 | TGCCAGTCATCACCACAGTA | 51477733 | 703 |
| | | Exon 21 | GAACCTGATACCGAGAAGAC | 51383758 | AAATGTCCAGGCTTTCCAGA | 51382987 | 772 |
| | | Exon 22 | GCCATAACAACTCACACGGG | 51367740 | TTGGCAGAAGGAATCATAGC | 51366885 | 856 |
| Second PCR | | 5'-UTR to exon 1 | TGTGGATGTTTTTGCTCGC | 52206503 | AGTAAACAGGTCCCGACGC | 52205586 | 918 |
| | | Exon 2 | GTGAACTGAGATTGTACCACTGC | 52168471 | CATATCCCTTATCAAAAATGCTT | 52167924 | 548 |
| | | Exon 3 | GAATGCTACCCAATTAAAGTGG | 52113285 | TTCAAAACCAAATACAGCCTC | 52112899 | 387 |
| | | Exon 4 | TGCCAAAGATGAAACACAGA | 52025601 | ACCCACAGATAATAGAGAACAAGA | 52025273 | 329 |
| | | Exon 5 | TGATGGTTCCTGATAGTAGTATTG | 52006775 | TGTCACACTAAAAATGTTGGG | 52006348 | 428 |
| | | Exon 6 | AAGGAAAGACTGAAAGTTAGCC | 51984688 | GAGCCTGAAGTTCCTATATGAT | 51984201 | 488 |
| | | Exon 7 | TTCTACTGTATCTTCACTCCACG | 51976953 | GCTTCTGCCTGATGTAGC | 51976541 | 413 |
| | | Exon 8 | GGCTGACTTTTCATTCTTTTT | 51964221 | CATCTTGCCGAAATCTCTCC | 51963831 | 391 |
| | | Exon 9 | TGTGATTTACGATGTGTACTTGG | 51880335 | GCAAGGTTGGGTGTGAGAG | 51879895 | 441 |
| | | Exon 10 | AAAATGGGAATAAAACTGTCTT | 51878507 | TCAGGATATGGAAGACTTAGCAC | 51877859 | 649 |
| | | Exon 11 | ACTGGTAACTGAAACTCAG | 51859069 | CAATTCCCTGAAAGCTAG | 51858628 | 442 |
| | | Exon 12 | TCAGTGCCCTTCAAATGTGT | 51834881 | ACCAAATAGAAATGCTCTTATAGA | 51834414 | 468 |
| | | Exon 13 | TCGGATGCTGTGTTGAAGTG | 51800982 | TGTGTAATGATAGGTCGTGTC | 51800543 | 440 |

**Table 1** Primer sequences for human *DPYD*

| Amplified and sequenced region | | Forward primer | | Reverse primer | | PCR product (bp) |
|---|---|---|---|---|---|---|
| | | Sequences (5' to 3') | Position[a] | Sequences (5' to 3') | Position[a] | |
| | Exon 14 | TGCAAATATGTGAGGAGGGACC | 51735287 | CAGCAAAGCAACTGGCAGATTC | 51734877 | 411 |
| | Exon 15 | GCTATCTTACCCTGCTATTTTC | 51667571 | TAGGTAGTGTGTGAAATCCAAGG | 51667107 | 465 |
| | Exon 16 | CCCCTTATGAGCACTGAGTAAAT | 51658821 | TAGTAACTATCCATACGGGGG | 51658440 | 382 |
| | Exon 17 | AGTCTAGGTGTAATACTGAGGAGG | 51591407 | ATCAAGTGCTCAACTGGAAACT | 51590986 | 422 |
| | Exon 18 | GTGAAGAACTTTGAGGAGAAGAC | 51590461 | CATCCTGTGCTGTCACTTGA | 51590026 | 436 |
| | Exon 19 | ATTTGTCCAGTGACGCTGTC | 51520048 | TCAGGTCTCTTCATAACTTGTCAG | 51519629 | 420 |
| | Exon 20 | GAGAAGTGAATTTGTTTGGAG | 51478265 | TTTGTTAGTGAGAATGTGAGATGG | 51477926 | 340 |
| | Exon 21 | AGTGGTCCAAAACAATGAGTG | 51383737 | TGCTTGCCAGTGTTCTAAAA | 51383221 | 517 |
| | Exon 22 | GGGTGTCATTTATTCTTTCTGTC | 51367723 | GGCTGATGAAATGGTATAAAAA | 51367033 | 691 |
| | Exon 23 | GTTGTCTCATAGTGTGGCTCCTC | 51364206 | TTTTTCACATAAGACAACTGGCA | 51363641 | 566 |
| Sequencing | 5'-UTR to exon 1 | TGTGGATGTTTTTGCTCGC | 52206503 | | | |
| | 5'-UTR to exon 1 | CGGACTGCTTTTACCTTTGC | 52206258 | CCAGAGAGCCAAGTGACAGC | 52205933 | |
| | 5'-UTR to exon 1 | CCCTAGTCTGCCTGTTTTCG | 52205987 | AGTAAACAGGTCCCGACGC | 52205586 | |
| | Exon 2 | GTGACAAAGTGAGAGAGACCGT | 52168436 | GCCTTACAATGTGTGGAGTGAG | 52168152 | |
| | Exon 3 | GAATGCTACCCAATTAAAGTGG | 52113285 | TTCAAAACCAAATACAGCCTC | 52112899 | |
| | Exon 4 | TGCCAAAGATGAAACACAGA | 52025601 | ACCCACAGATAATAGAGAACAAGA | 52025273 | |
| | Exon 5 | TGATGGTTCCTGATAGTAGTATTG | 52006775 | TGTCACACTAAAAATGTTGGG | 52006348 | |
| | Exon 6 | AAAATATGTTTGAGGATGTAAGC | 51984560 | GAGCCTGAAGTTCCTATATGAT | 51984201 | |
| | Exon 7 | TTCTACTGTATCTTCACTCCACG | 51976953 | GCTTCTGCCTGATGTAGC | 51976541 | |
| | Exon 8 | GGCTGACTTTTCATTCTTTTT | 51964221 | CATCTTGCCGAAATCTCTCC | 51963831 | |
| | Exon 9 | TGTGATTTACGATGTGTACTTGG | 51880335 | GCAAGGTTGGGTGTGAGAG | 51879895 | |
| | Exon 10 | AAAATGGGAATAAAACTGTCTT | 51878507 | TTCATCTCCTAAAATCTGTTGG | 51878109 | |
| | Exon 11 | ACTGGTAACTGAAACTCAG | 51859069 | CAATTCCCTGAAAGCTAG | 51858628 | |
| | Exon 12 | TCAGTGCCCTTCAAATGTGT | 51834881 | GAGTATCAAAAATAAATGAAGCAC | 51834439 | |
| | Exon 13 | TCGGATGCTGTGTTGAAGTG | 51800982 | TGTGTAATGATAGGTCGTGTC | 51800543 | |
| | Exon 14 | TGCAAATATGTGAGGAGGGACC | 51735287 | CAGCAAAGCAACTGGCAGATTC | 51734877 | |
| | Exon 15 | GCTATCTTACCCTGCTATTTTC | 51667571 | TAGGTAGTGTGTGAAATCCAAGG | 51667107 | |
| | Exon 16 | CCCCTTATGAGCACTGAGTAAAT | 51658821 | TAGTAACTATCCATACGGGGG | 51658440 | |
| | Exon 17 | AGTCTAGGTGTAATACTGAGGAGG | 51591407 | ATCAAGTGCTCAACTGGAAACT | 51590986 | |
| | Exon 18 | GTGAAGAACTTTGAGGAGAAGAC | 51580461 | CATCCTGTGCTGTCACTTGA | 51590026 | |
| | Exon 19 | ATTTGTCCAGTGACGCTGTC | 51520048 | CGAATCTATTTTTTTTTTGTCAC | 51519715 | |
| | Exon 20 | GAGAAGTGAATTTGTTTGGAG | 51478265 | TTTGTTAGTGAGAATGTGAGATGG | 51477926 | |
| | Exon 21 | TATCTTCCCATTTTTCTCTTCTC | 51383644 | TGCCAGTGTTCTAAAAAGTATAAA | 51383225 | |
| | Exon 22 | GTATAAAAACAGGAAAATGCTGA | 51367510 | ATAAGGGTGACAGGACAGAAAG | 51367125 | |
| | Exon 23 | GTTGTCTCATAGTGTGGCTCCTC | 51364206 | TATTTGTTTTAATTTGGAAAGAG | 51363821 | |

[a] Nucleotide position of the 5' end of each primer on NT_032977.7

Foster City, CA) with the primers listed in "sequencing" of Table 1. Excess dye was removed with a DyeEx96 kit (Qiagen, Hilden, Germany). The eluates were analyzed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems). All novel SNPs were confirmed by sequencing of PCR products generated from new genomic DNA amplifications. The genomic and cDNA sequences of *DPYD* obtained from GenBank (NT_032977.7 and NM_000110.2, respectively) were used as reference sequences. SNP positions were numbered based on the cDNA sequence, and adenine of the translational initiation site in exon 1 was numbered +1. For intronic polymorphisms, the position was numbered from the nearest exon.

### Linkage disequilibrium (LD) and haplotype analyses

Hardy-Weinberg equilibrium and LD analyses were performed by SNPAlyze software (Dynacom Co., Yokohama, Japan), and pairwise LD parameters between variations were obtained as the ID'I and rho square ($r^2$) values. Some haplotypes were unambiguously identified from subjects with homozygous variations at all sites or a heterozygous variation at only one site. Diplotype configurations were inferred by LDSUPPORT software, which determines the posterior probability distribution of the diplotype for each subject based on the estimated haplotype frequencies (Kitamura et al. 2002). Although the nomenclature for nonsynonymous *DPYD* alleles (*DPYD*1* to *DPYD*13*) have been already publicized (McLeod et al. 1998; Collie-Duguid et al. 2000; Johnson et al. 2002), several reported alleles remain unassigned. To avoid confusion with the previous *DPYD* allele nomenclature, our block haplotypes in this study were tentatively defined by using '#' instead of '*'. A group of haplotypes without any amino acid change is designated as *#1*, and the haplotype groups bearing already defined alleles, *DPYD*5* (Ile543Val), *DPYD*6* (Val732Ile), *DPYD*9* (Cys29Arg) and *DPYD*11* (Val335Leu), were numbered by using the corresponding Arabic numerals, *#5*, *#6*, *#9*, and *#11*, respectively. Other haplotypes with known nonsynonymous SNPs such as 496A>G (Met166Val) or with the novel nonsynonymous SNP were represented by '#' plus amino acid positions followed by variant residues (for example, *#166V*). Subtypes within each haplotype group were consecutively named with small alphabetical letters depending on their frequencies. Haplotypes ambiguously inferred in only one patient were indicated in the Fig. 3 legend. Combinations of block haplotypes were analyzed by Haploview software (http://www.broad.mit.edu/mpg/haploview/index.php) (Barrett et al. 2005), and the long-range (whole gene) haplotypes spanning all blocks were inferred by Hapblock software (www.cmb.usc.edu/msms/HapBlock/) (Zhang et al. 2005).

Typing data on *DPYD* from unrelated 44 Japanese and 30 Caucasian trios were also obtained from the HapMap project (HapMap release 19: http://www.hapmap.org/). The LD profiles and haplotypes of the HapMap data were obtained by Marker beta in Gmap Net (http://www.gmap.net/marker) using its four (1254711, 1254712, 1254713, and 1254714) and six (1166276, 1166277, 1166278, 1166279, 1166280, and 1166281) datasets covering *DPYD* genomic regions for Japanese and Caucasians, respectively.

### Drawing of protein structures

The coordinate data (1gth) of the crystal structure of pig DPD (Dobritzsch et al. 2002) was obtained from the Protein Data Bank. Protein Explorer (http://proteinexplorer.org) (Martz 2002) was used to display the structural features of pig DPD and depict three-dimensional views.

## Results

### *DPYD* variations found in a Japanese population

We identified 55 variations, including 38 novel ones by sequencing the promoter regions (up to 613 bp upstream from the translational initiation site), all 23 exons and their flanking regions of *DPYD* from 341 Japanese subjects (Table 2). The distribution of the variations consisted of 4 in the 5' flanking region, 21 (5 synonymous and 16 nonsynonymous ones) in the coding exons (Fig. 1) and 30 in the introns. Since we did not find any significant differences in allele frequencies between healthy volunteers and cancer patients ($P > 0.05$ by $\chi^2$ test or Fisher's exact test) except for one variation, IVS14 + 19C>A, ($P = 0.027$ by Fisher's exact test); the data for all subjects were analyzed as one group. All detected variations except for 451A>G (Asn151Asp) and IVS13 + 40G>A were in Hardy-Weinberg equilibrium ($P \geq 0.24$).

Thirteen novel variations in the coding region (enclosed by a square in Fig. 1) contain four synonymous SNPs, 474T>C (Phe158Phe), 639C>T (Asp213Asp), 1752A>G (Thr584Thr), and 2424T>C (Ser808Ser) and nine nonsynonymous SNPs, 29C>A (Ala10Glu), 325T>A (Tyr109Asn), 451A>G (Asn151Asp), 733A>T (Ile245Phe), 793G>A (Glu265Lys), 1543G>A (Val515Ile), 1572T>G (Phe524-Leu), 1666A>C (Ser556Arg), and 2678A>G (Asn893Ser). 451A>G (Asn151Asp), 325T>A (Tyr109Asn), and 2678A>G (Asn893Ser) were found at frequencies of 0.009, 0.003 and 0.003, respectively. The others were detected as single heterozygotes (allele frequencies = 0.0015).

**Table 2** Summary of *DPYD* SNPs detected in a Japanese population

| SNP ID | | Location | Position | | Nucleotide change and flanking sequences (5′ to 3′) | Amino acid change | Reported alleles | Allele frequency (341 subjects) |
|---|---|---|---|---|---|---|---|---|
| This study | dbSNP (NCBI) | | NT_032977.7 | From the translational initiation site or from the end of nearest exon | | | | |
| MPJ6_DPD001[a] | | 5′-Flank | 52206480 | –609 | TTGCTCGCCTCCC/TTCCCCTCCCCGC | | | 0.021 |
| MPJ6_DPD002[a] | | 5′-Flank | 52206348 | –477 | TTGAGGAGTTCCT/GGAAAATGCAGTT | | | 0.026 |
| MPJ6_DPD003[a] | | 5′-Flank | 52206137 | –266 | CTCCCTCCCTCCC/ATTCTGCTTGCAG | | | 0.045 |
| MPJ6_DPD004[a] | | 5′-Flank | 52206114 | –243 | AGGCTGGGGCGCG/AGAGCGGGCTGAA | | | 0.0059 |
| MPJ6_DPD005[a] | | Exon 1 | 52205843 | 29 | GTAAGGACTCGGC/AGGACATCGAGGT | Ala10Glu | | 0.0015 |
| MPJ6_DPD006[g] | rs1801265 | Exon 2 | 52168278 | 85 | CATGCAACTCTGT/CGTTCCACTTCGG | Cys29Arg | *9 | 0.029 |
| MPJ6_DPD007[a] | | Intron 2 | 52168055 | IVS2 + 158 | TTTGAAAGTGTAT/CTTTTAATTACAC | | | 0.0015 |
| MPJ6_DPD008[a] | | Intron 3 | 52113040 | IVS3 + 23 | GTCACCATAGCAA/GCAGTCACAGATG | | | 0.0029 |
| MPJ6_DPD009[a] | | Exon 5 | 52006617 | 325 | ATTTTGCAGAACT/AATTATGGAGCTG | Tyr109Asn | | 0.0029 |
| MPJ6_DPD010[a] | | Exon 5 | 52006491 | 451 | GAGGGACCCATTA/GATATTGGTGGAT | Asn151Asp | | 0.0088 |
| MPJ6_DPD011[a] | | Exon 5 | 52006468 | 474 | ATTGCAGCAATTT/CGCTACTGAGGTA | Phe158Phe | | 0.0044 |
| MPJ6_DPD012[a] | | Intron 5 | 51984611 | IVS5–115 | CATATTAATACTG/AAAAATGTACTGC | | | 0.021 |
| MPJ6_DPD013[g] | rs2297595 | Exon 6 | 51984484 | 496 | GTATTCAAAGCAA/GTGAGTATCCCAC | Met166Val | | 0.022 |
| MPJ6_DPD014[a] | | Exon 6 | 51984341 | 639 | GGGGTACTCTGAC/TATCACTATATTT | Asp213Asp | | 0.0088 |
| MPJ6_DPD015[a] | | Exon 7 | 51976695 | 733 | GTGAATTTTGAGA/TTTGAGCTAATGA | Ile245Phe | | 0.0015 |
| MPJ6_DPD016[a] | | Intron 7 | 51976602 | IVS7 + 64 | CTCTACACTAAAG/TATTAACAGCAAA | | | 0.0015 |
| MPJ6_DPD017[a] | | Exon 8 | 51964101 | 793 | CTTTCAGTGAATG/AAAATGACTCTTA | Glu265Lys | | 0.0015 |
| MPJ6_DPD018[a] | | Intron 8 | 51963953 | IVS8 + 91 | TTCAGACATTTTC/TGTGATGAAAGTT | | | 0.0088 |
| MPJ6_DPD019[a] | | Intron 9 | 51878456 | IVS9–120 | TTTGATAGTGACA/TCTTCATCCTGGA | | | 0.0029 |
| MPJ6_DPD020[b] | | Exon 10 | 51878292 | 1003 | ATACGGGGAGTCG/TTGATTGTACTTG | Val335Leu | *11 | 0.0015 |
| MPJ6_DPD021[a] | | Intron 10 | 51878143 | IVS10 + 24 | CCATCAGAAAATA/GTGGAGTTGTACT | | | 0.0015 |
| MPJ6_DPD022[c] | | Intron 10 | 51858934 | IVS10-15 | TTTCTTCTCTGTT/CCTGTTTTGTTTT | | | 0.018 |
| MPJ6_DPD023[a] | | Intron 12 | 51800901 | IVS12-11 | AAGTATTGGTTTG/ATATTTTGCAGTC | | | 0.038 |
| MPJ6_DPD024[a] | | Intron 12 | 51800899 | IVS12-9 | GTATTGGTTTGTA/GTTTTGCAGTCAC | | | 0.0073 |
| MPJ6_DPD025[a] | | Exon 13 | 51800872 | 1543 | TATGGAGCTTCCG/ATTTCTGCCAAGC | Val515Ile | | 0.0015 |
| MPJ6_DPD026[a] | | Exon 13 | 51800843 | 1572 | ACTACCCCTCTTT/GTACACTCCTATT | Phe524Leu | | 0.0015 |
| MPJ6_DPD027[g] | rs1801159 | Exon 13 | 51800788 | 1627 | GGATTGAAGTTTA/GTAAATCCTTTTG | Ile543Val | *5 | 0.283 |
| MPJ6_DPD028[a] | | Exon 13 | 51800749 | 1666 | ACTCCAGCCACCA/CGCACATCAATGA | Ser556Arg | | 0.0015 |
| MPJ6_DPD029 | rs2786783 | Intron 13 | 51800636 | IVS13 + 39 | AGAAATGTCTATC/TATATATTTTAAT | | | 0.283 |
| MPJ6_DPD030 | rs2811178 | Intron 13 | 51800635 | IVS13 + 40 | GAAATGTCTATCG/ATATATTTTAATT | | | 0.179 |
| MPJ6_DPD031[a] | | Intron 13 | 51735220_51735219 | IVS13-47_-48 | ATAAAGATTATA–/TAGCTTTTCTTTGT | | | 0.0015 |
| MPJ6_DPD032[a] | | Exon 14 | 51735161 | 1752 | GGACATTGTGACA/GAATGTTTCCCCC | Thr584Thr | | 0.0015 |
| MPJ6_DPD033[f] | | Exon 14 | 51735139 | 1774 | CCCAGAATCATCC/TGGGGAACCACCT | Arg592Trp | | 0.0015 |
| MPJ6_DPD034[g] | rs17376848 | Exon 14 | 51735017 | 1896 | AAAGGCTGACTTT/CCCAGACAACGTA | Phe632Phe | | 0.139 |
| MPJ6_DPD035[a] | | Intron 14 | 51734989 | IVS14 + 19 | GTGATTTAACATC/ATAAAACAAGAGA | | | 0.0088 |
| MPJ6_DPD036[a] | | Intron 14 | 51734908 | IVS14 + 100 | TTAATGTGTATAT/GTTTATTAAAGAA | | | 0.0015 |
| MPJ6_DPD037[a] | | Intron 14 | 51667533 | IVS14-123 | GATTTATTTTTTC/AACAGTTTGAAAA | | | 0.155 |
| MPJ6_DPD038[a] | | Intron 14 | 51667431 | IVS14-21 | TGAACTTATATTC/ATTTTGTTTTTCT | | | 0.0015 |
| MPJ6_DPD039[d] | | Intron 15 | 51667267 | IVS15 + 75 | TAAAGAGCTGCCA/GTGAGAAATAATA | | | 0.155 |
| MPJ6_DPD040[a] | | Intron 16 | 51591373 | IVS16-127 | GGAATTTGAGAAA/GTATATCATGTAG | | | 0.0015 |

**Table 2** continued

| SNP ID | | Location | Position | | Nucleotide change and flanking sequences (5′ to 3′) | Amino acid change | Reported alleles | Allele frequency (341 subjects) |
|---|---|---|---|---|---|---|---|---|
| This study | dbSNP (NCBI) | | NT_032977.7 | From the translational initiation site or from the end of nearest exon | | | | |
| MPJ6_DPD041[g] | rs7556439 | Intron 16 | 51591340 | IVS16-94 | CAAGTTGGATTTG/TTCTTGCACGTCT | | | 0.378 |
| MPJ6_DPD042[a] | | Intron 17 | 51591092 | IVS17 + 34 | GTTGCCCGCTATT/-GTAAATATTGGC | | | 0.0015 |
| MPJ6_DPD043[a] | | Intron 17 | 51591079 | IVS17 + 47 | GTAAATATTGGCC/TCACATTATGTAG | | | 0.0015 |
| MPJ6_DPD044 | rs1801160 | Exon 18 | 51590313 | 2194 | GGTGCCAATGGCG/ATTACAGCCACCA | Val732Ile | *6 | 0.015 |
| MPJ6_DPD045[g] | rs12137711 | Intron 18 | 51519982 | IVS18-39 | TATACTCAAGTGG/ATCAGTGTGCTAA | | | 0.032 |
| MPJ6_DPD046[c] | | Exon 19 | 51519940 | 2303 | TTTGTGTAGGGAC/AAGCAATCAGACC | Thr768Lys | | 0.028 |
| MPJ6_DPD047[a] | | Exon 19 | 51519819 | 2424 | GTTTCTCCATAGT/CGGTGCTTCCGTC | Ser808Ser | | 0.0029 |
| MPJ6_DPD048[a] | | Exon 21 | 51383526 | 2678 | TCATAGCAGAAAA/GCAAGATTAGACT | Asn893Ser | | 0.0029 |
| MPJ6_DPD049[a] | | Intron 21 | 51383358 | IVS21 + 80 | GTTTATTTACTGC/GTTAAATGTATCA | | | 0.0015 |
| MPJ6_DPD050[a] | | Intron 21 | 51383325 | IVS21 + 113 | GTTTTAGAATTAT/AAATGAAAGTTTT | | | 0.0015 |
| MPJ6_DPD051[g] | rs11165777 | Intron 21 | 51383302 | IVS21 + 136 | TTAAAAACATCTG/CTCCATGGTGAAA | | | 0.0029 |
| MPJ6_DPD052[a] | | Intron 21 | 51383276 | IVS21 + 162 | CTGCATTTAAATT/GATAAAATAACCT | | | 0.0029 |
| MPJ6_DPD053[a] | | Intron 22 | 51367150 | IVS22 + 129 | TTCTGCAACAGTA/GCATCTTTCTGTC | | | 0.0073 |
| MPJ6_DPD054[g] | rs290855 | Intron 22 | 51364164 | IVS22-69 | GAGAAAAATGTTG/AACGCTAAAATGG | | | 0.0029 |
| MPJ6_DPD055[g] | rs17116357 | Intron 22 | 51364153 | IVS22-58 | TAACGCTAAAATG/CGGGACATTGTTG | | | 0.0029 |

[a] Novel variations detected in this study

[b] Kouwaki et al. 1998

[c] Collie-Duguid et al. 2000

[d] Seck et al. 2005

[e] Ogura et al. 2005

[f] Cho et al. 2007

[g] Variations overlapping with the HapMap project

In the 5′ flanking region, all four detected SNPs (-609C>T, -477T>G, -266C>A, -243G>A) were newly found at relatively high allele frequencies (0.006–0.05). However, these SNPs were not located near the proposed *cis*-regulatory promoter elements (Shestopal et al. 2000). The remaining 21 novel variations were found in intronic regions. Of these SNPs, IVS5–115G>A, IVS12–11G>A, and IVS14-123C>A were detected with allele frequencies of 0.021, 0.038, and 0.155, respectively, but others were rare (<0.01). They were not located in the exon-intron splicing junctions or branch sites.

Seventeen variations were already reported. The ID numbers in the dbSNP databases or references for these SNPs are described in Table 2. The well-known nonsynonymous SNPs, 1627A>G (**5*, Ile543Val), 2194G>A (**6*, Val732Ile), 85T>C (**9*, Cys29Arg), and 1003G>T (**11*, Val335Leu), were found in this study at allele frequencies of 0.283, 0.015, 0.029, and 0.0015, respectively. The allele frequencies of two reported SNPs, 496A>G (Met166Val) and 2303C>A (Thr768Lys), were 0.022 and 0.028, respectively. Recently, 1774C>T (Arg592Trp) was reported from a Korean population (Cho et al. 2007), and its allele frequency was 0.0015 in this study. Nine intronic variations, IVS10-15T>C, IVS13 + 39C>T, IVS13 + 40 G>A, IVS15 + 75A>G, IVS16-94G>T, IVS18-39G>A, IVS21 + 136G>C, IVS22-58G>C, and IVS22-69G>A, and one synonymous variation, 1896T>C (Phe632Phe), were found with various allele frequencies (0.003–0.378, Table 2). The variations previously detected in Japanese (Kouwaki et al. 1998; Yamaguchi et al. 2001; Ogura et al. 2005), 62G>A (Arg21Gln, **12*), 74G>A (His25Arg), 812delT (Leu271X), 1097G>C (Gly366Ala), 1156G>T (Glu386X, **12*), and 1714C>G (Leu572Val), were not found in our study. This might be due to their low frequencies.

## Linkage disequilibrium (LD) analysis and haplotype block partition

LD analysis was performed by $r^2$ and |D′| using 18 SNPs (allele frequency ≥0.01) (Fig. 2). Strong linkages were observed in four pairs of SNPs: between -477T>G and 85T>C (Cys29Arg) ($r^2$ = 0.7025), between 496A>G (Met166Val) and IVS10-15T>C ($r^2$ = 0.7964), between 1627A>G (Ile543Val) and IVS13 + 39C>T ($r^2$ = 1.0), and between IVS14-123C>A and IVS15 + 75A>G ($r^2$ = 1.0). In addition, two known rare SNPs, IVS22-69G>A (rs290855) and IVS22-58G>C (rs17116357), were perfectly linked ($r^2$ = 1.0) (data not shown). As for |D′| values, only 43 pairs (28%) out of 153 pairs gave |D′| = 1.0, indicating that a number of recombinations had occurred within this gene. This is not surprising because *DPYD* is a huge gene of at least 950 kb in length with 3 kb of coding sequences. However, it was difficult to estimate past recombination events in *DPYD* from our data alone because our variations were mostly limited to exons and surrounding introns.

To define haplotype blocks, we utilized the HapMap data because SNPs were comprehensively genotyped with an average density of 1 SNP per 1.8 kb. Of 1,002 variations of *DPYD* genotyped by the HapMap project, 474 SNPs were polymorphic for 44 unrelated Japanese subjects. When the LD profiles for Japanese were obtained by Marker using the HapMap data, strong LD (|D′| > 0.75) clearly decays within introns 11, 12, 13, 14, 16, 18, and 20 (data not shown), suggesting that recombination had occurred in these regions. Based on these findings, the SNPs detected in our study were divided into six haplotype blocks (Figs. 1, 2). Block 1, the largest block, ranges from the 5′-untranslated region (5′-UTR) to intron 10 (347 kb), and includes 22 variations. Block 2 includes eight variations from IVS12-11G>A in intron 12 to IVS13 + 40G>A in intron 13. Block 3 includes six variations from IVS13-47_48insTA in intron 13 to IVS14 + 100T>G in intron 14. Block 4 contains only three SNPs, IVS14-123C>A, IVS14-21C>A and IVS15 + 75A>G, and ranges from intron 14 to intron 15. Block 5 consists of IVS16-94G>T and four rare variations from intron 16 to exon 18. Although the HapMap data showed a decline in LD in intron 20, we defined a block ranging from intron 18 to intron 22 as block 6 because only rare variations (allele frequencies <0.01) were detected downstream of intron 20 (exon 21, intron 21, and intron 22). The block partitioning based on the HapMap data fitted our SNPs well: more than 70% of SNP pairs in each block (block 1–6) gave pair-wise |D′| values greater than 0.8 (Fig. 2).

## Haplotype estimation

Using 22, 8, 6, 3, 5, and 11 variations in blocks 1 to 6, 23 (block 1), 8 (block 2), 7 (block 3), 3 (block 4), 6 (block 5), and 11 (block 6) haplotypes were identified or inferred (Fig. 3). Probabilities of diplotype configurations in all six blocks were 100% for over 97% of the subjects. To discriminate our block haplotypes from the previously assigned alleles or haplotypes (*DPYD*1* to **13*), the mark, #, was used to indicate block haplotypes.

In block 1, the most dominant haplotype without any variation was #*1a* (0.818 in frequency), followed by #*1b* (0.045), #*9c* (0.022), and #*1c* (0.021). As suggested by LD (Fig. 2), #*9c*, the major subtype of the #*9* group bearing 85T>C (Cys29Arg), also harbored -477T>G in the 5′-UTR. Known nonsynonymous SNP, 496A>G (Met166Val), was assigned to three haplotypes, #*9d*, #*166Va*, and #*166Vb*.

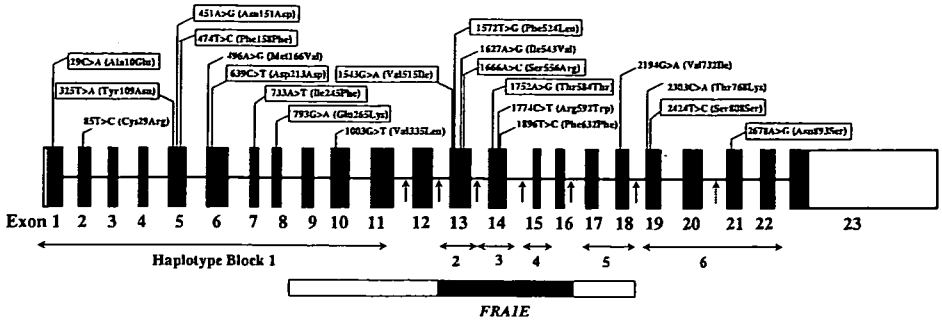



**Fig. 1** Twenty-one variations detected in the coding exons are depicted in the schematic diagram of the *DPYD* gene. Fourteen novel variations are enclosed by *squares*. The recombination spots were estimated based on the LD profiles obtained from Japanese data in the HapMap project and indicated by *arrows*. The borders (between introns 8 and 18 of the *DPYD*) and core region (between introns 12 and 16) of *FRA1E* identified by Hormozian et al. (2007) are indicated as an *open* and *closed box*, respectively

In block 2, four haplotypes, #*1a* (0.529), #*5a* (0.245), #*1b* (0.176), and #*5b* (0.038), were major in Japanese and accounted for 99% of all inferred haplotypes. Two subtypes of the #*5* group, #*5a* and #*5b*, both of which harbored Ile543Val (**5*) and IVS13 + 39C>T, were distinguished by a novel intronic SNP, IVS12-11G>A.

As for block 3, in addition to #*1a* (0.848), #*1b* harboring the synonymous SNP, 1896T>C (Phe632Phe), was found at a relatively high frequency (0.138).

Block 4 is simple and comprises only three haplotypes, #*1a* (0.845), #*1b* (0.154) and #*1c* (0.0015). The second frequent haplotype, #*1b*, harbored perfectly linked SNPs, IVS14-123C>A and IVS15 + 75A>G.

Block 5 contained IVS16-94G>T, the most frequent SNP among the 55 SNPs found in this study, which was assigned to #*1b* with a frequency of 0.374. This block also contained the known nonsynonymous SNP, 2194G>A (Val732Ile, **6*), which was assigned to #*6a* (0.015).

In block 6, the most dominant haplotype was #*1a* (0.915). It was followed by #*1b* (0.032) with IVS18-39G>A and #*768K* (0.028) with 2303C>A (Thr768Lys).

The HapMap data include nine SNPs that we detected (Table 2). Of them, six, 85T>C (rs1801265), 496A>G (rs2297595), 1627A>G (rs1801159), 1896T>C (rs17376848), IVS16-94G>T (rs7556439) and IVS18-39G>A (rs12137711), were suitable for haplotype tagging SNPs (htSNPs) to capture the block haplotypes, block 1 #*9*, block 1 #*166V*, block 2 #*5*, block 3 #*1b*, block 5 #*1b*, and block 6 #*1b*, respectively. IVS21 + 136G>C (rs11165777) and IVS22-69G>A (rs290855)/IVS22-58G>C (rs1711 6357), were the marker SNPs for block 6 #*1e* and #*1f*, respectively, but very rare (allele frequencies = 0.003) in Japanese. The six SNPs, especially 85T>C (rs1801265) and 496A>G (rs2297595), were in strong LD ($r^2 > 0.8$) with other HapMap SNPs in Japanese (Table 3), indicating that many HapMap SNPs were concurrently linked on the same haplotypes.

Next, the combinations of block haplotypes (inter-block haplotypes) were analyzed focusing on the haplotypes with frequencies of >0.01 in each block (Fig. 4). Between blocks 1 and 2, both #*1a* and #*1b* in block 1 were complicatedly associated with various haplotypes in block 2. It should be noted that #*9c* in block 1 was linked either with block 2 #*1b* (0.016 in absolute frequency) or with block 2 #*5a* (0.006, not shown in Fig. 4). #*1c* in block 1 was completely linked with block 2 #*1a*. #*151D* in block 1 (not shown in Fig. 4), which was a rare haplotype (0.009) harboring 451A>G (Asn151Asp), was completely linked with #*5a* in block 2.

Between blocks 2 and 3, both #*5b* and #*1b* in block 2 were mostly linked with #*1a* in block 3, whereas both #*1a* and #*5a* in block 2 were complicatedly linked with #*1a*, #*1b*, or other rare haplotypes such as #*1c* (not shown in Fig. 4) in block 3. Between blocks 3 and 4 and between blocks 4 and 5, no strong associations of block haplotypes were observed except for the linkage of block 5 #*6a* to block 4 #*1a*. Between blocks 5 and 6, most of #*1b* and all of #*6a* in block 5 were linked with #*1a* in block 6. Although #*1a* in block 6 was associated with various haplotypes in block 5, #*1b* in block 6 was completely linked with #*1a* in block 5.

Among the six blocks, the following combinations were major: #*1a* (block 1)–#*1a* (block 2) –#*1a* (block 3)–#*1a* (block 4)–#*1a* (block 5)–#*1a* (block 6) (0.239 in frequency), #*1a*–#*5a*–#*1a*–#*1a*–#*1b*–#*1a* (0.081), #*1a*–#*1a*–#*1a*–#*1a*–#*1b*–#*1a* (0.075), #*1a*–#*5a*–#*1a*–#*1a*–#*1a*–#*1a* (0.070), #*1a*–#*1b*–#*1a*–#*1a*–#*1a*–#*1a* (0.060) and #*1a*–#*1a*–#*1b*–#*1a*–#*1a*–#*1a* (0.051).

## Ethnic differences in distributions of *DPYD* SNPs and haplotypes

We compared SNP and haplotype distributions in Japanese with those in other ethnic groups reported in the literature

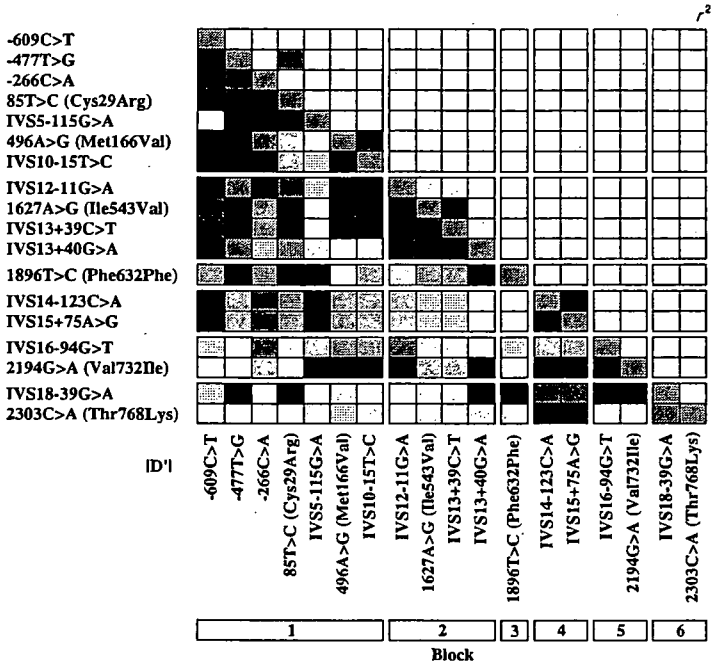



**Fig. 2** Linkage disequilibrium (*LD*) analysis of *DPYD*. Pairwise LD between 18 common SNPs (>0.01 in allele frequencies) is expressed as $r^2$ (*upper*) and |D'| (*lower*) by a 10-graded *blue color*. The *denser color* indicates higher linkage. The haplotype block partition based on LD measure |D'| of HapMap data in Japanese is also indicated

or HapMap project. Notably, IVS14 + 1G>A (*2), 1897delC (Pro633GlnfsX5, *3), 1601G>A (Ser534Asn, *4), 295_298delTCAT (Phe100SerfsX15, *7), 703C>T (Arg235Trp, *8), 2983G>T (Val995Phe, *10), 62G>A (Arg21Gln, *12), 1156G>T (Glu386X, *12), and 1679T>G (Ile560Ser, *13) were not found in this study. Furthermore, several SNPs showed marked differences in allele frequencies among Japanese and other ethnic groups (Table 4).

The allele frequency of 85T>C (Cys29Arg, *9), the tagging SNP for block 1 #9, was quite different between Asians and Caucasians. Its allele frequency in Japanese (0.029 in this study) and Taiwanese (0.022) (Hsiao et al. 2004) was much lower than that in Caucasians (0.185–0.194) (Seck et al. 2005; Morel et al. 2006).

The SNP 496A>G (Met166Val) in block 1 is found at a lower allele frequency in Japanese (0.022) than in Caucasians (0.080) (Seck et al. 2005). Seck et al. (2005) inferred two haplotypes harboring 496A>G (Met166Val) from 157 Caucasians: *hap5* (#9d in this study) harboring additional 85T>C (Cys29Arg) and IVS10-15T>C and *hap11* concurrently harboring IVS10-15T>C alone with frequencies of 0.040 and 0.014, respectively. In our haplotype analysis, #166Va (0.012) corresponding to *hap11* (0.014) was found with a similar frequency in Japanese, whereas the frequency of #9d (0.006) was much lower than that of the corresponding haplotype, *hap5* (0.040) in Caucasians.

1627A>G (Ile543Val, *5) in block 2 was found with comparable allele frequencies among Japanese (0.283 in this study), Caucasians (0.14-0.275) (Seck et al. 2005; Ridge et al. 1998a), African-Americans (0.227) (Wei et al. 1998), and Taiwanese (0.210–0.283) (Wei et al. 1998; Hsiao et al. 2004).

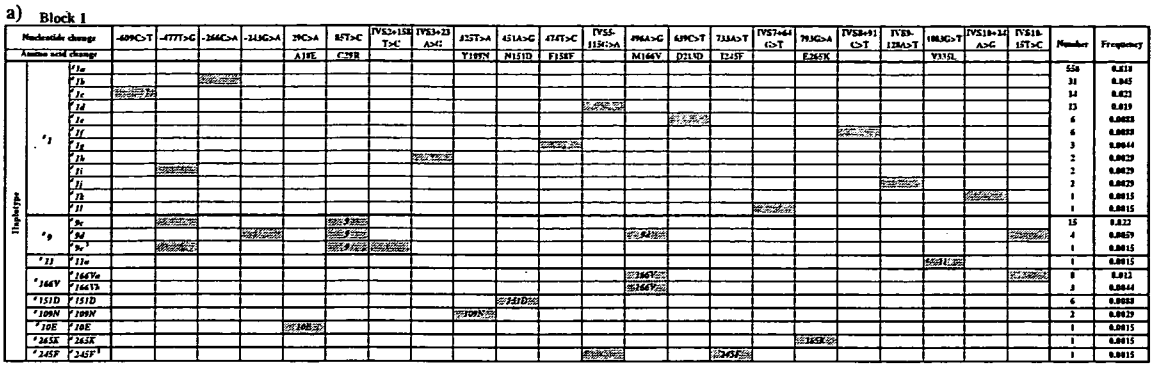
The allele frequency (0.015) of 2194G>A (Val732Ile, *6) in block 5 in our Japanese population is slightly lower than that previously reported in Caucasians (0.022-0.058) (Seck et al. 2005; Ridge et al. 1998a) and Finish (0.067) (Wei et al. 1998), but is comparable to that in Taiwanese (0.012-0.014) (Wei et al. 1998; Hsiao et al. 2004) and African-Americans (0.019) (Wei et al. 1998).

Ethnic differences in the allele frequencies were also observed with synonymous and intronic variations (Table 4). The allele frequency of 1896T>C (Phe632Phe), which tags block 3 #1b, was higher in Japanese (0.139 in this study) than in Caucasians (0.035) (Seck et al. 2005). *Hap13* assigned in 157 Caucasians by Seck et al. (2005) is the counterpart of block 3 #1b, and its frequency (0.012) was much lower than that in Japanese (0.138).

In contrast, IVS10-15T>C linked to 85T>C (*9) or 496A>G (#166V) within block 1 showed a lower allele frequency in Japanese (0.018) than in Caucasians (0.127). Seck et al. (2005) assigned *hap7* as the haplotype containing IVS10-15T>C alone with a haplotype frequency of 0.03 in Caucasians. In Japanese, however, the corresponding haplotype was not found.

Allele frequencies of IVS18-39G>A and IVS22-69G>A, which are tagging SNPs for block 6 #1b and #1f, respectively, are lower in Japanese (0.032 and 0.003, respectively) than in Caucasians (0.105 and 0.183, respectively).

a) Block 1



b) Block 2

| Nucleotide change | | | IVS12-11G>A | IVS12-9A>G | 1543G>A | 1572T>G | 1627A>G | 1666A>C | IVS13+39 C>T | IVS13+40 G>A | Number | Frequency |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amino acid change | | | | | V515I | F524L | I543V | S556R | | | | |
| Haplotype | *1 | *1a | | | | | | | | | 361 | 0.529 |
| | | *1b | | | | | | | | | 120 | 0.176 |
| | | *1c | | | | | | | | | 5 | 0.0073 |
| | *5 | *5a | | | | | | | | | 167 | 0.245 |
| | | *5b | | | | | | | | | 26 | 0.038 |
| | *515I | *515I | | | | | | | | | 1 | 0.0015 |
| | *524L | *524L | | | | | | | | | 1 | 0.0015 |
| | *556R | *556R§ | | | | | | | | | 1 | 0.0015 |

c) Block 3

| Nucleotide change | | | IVS13-47_48insTA | 1752A>G | 1774C>T | 1896T>C | IVS14+19 C>A | IVS14+100 T>G | Number | Frequency |
|---|---|---|---|---|---|---|---|---|---|---|
| Amino acid change | | | | T584T | R592W | F632F | | | | |
| Haplotype | *1 | *1a | | | | | | | 578 | 0.848 |
| | | *1b | | | | | | | 94 | 0.138 |
| | | *1c | | | | | | | 6 | 0.0088 |
| | | *1d | | | | | | | 1 | 0.0015 |
| | | *1e | | | | | | | 1 | 0.0015 |
| | | *1f§ | | | | | | | 1 | 0.0015 |
| | *592W | *592W | | | | | | | 1 | 0.0015 |

d) Block 4

| Nucleotide change | | | IVS14-123C>A | IVS14-21C>A | IVS15+75 A>G | Number | Frequency |
|---|---|---|---|---|---|---|---|
| Amino acid change | | | | | | | |
| Haplotype | *1 | *1a | | | | 576 | 0.845 |
| | | *1b | | | | 105 | 0.154 |
| | | *1c§ | | | | 1 | 0.0015 |

e) Block 5

| Nucleotide change | | | IVS16-127A>G | IVS16-94G>T | IVS17+34delT | IVS17+47C>T | 2194G>A | Number | Frequency |
|---|---|---|---|---|---|---|---|---|---|
| Amino acid change | | | | | | | V732I | | |
| Haplotype | *1 | *1a | | | | | | 414 | 0.607 |
| | | *1b | | | | | | 255 | 0.374 |
| | | *1c§ | | | | | | 1 | 0.0015 |
| | | *1d§ | | | | | | 1 | 0.0015 |
| | | *1e§ | | | | | | 1 | 0.0015 |
| | *6 | *6a | | | | | *6 | 10 | 0.015 |

f) Block 6

| Nucleotide change | | | IVS18-39G>A | 2303C>A | 2424T>C | 2678A>G | IVS21+80 C>G | IVS21+113 T>A | IVS21+136 G>C | IVS21+162 T>G | IVS22+129 A>G | IVS22-69 G>A | IVS22-58 G>C | Number | Frequency |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Amino acid change | | | | T768K | S808S | N893S | | | | | | | | | |
| Haplotype | *1 | *1a | | | | | | | | | | | | 624 | 0.915 |
| | | *1b | | | | | | | | | | | | 22 | 0.032 |
| | | *1c | | | | | | | | | | | | 5 | 0.0073 |
| | | *1d | | | | | | | | | | | | 2 | 0.0029 |
| | | *1e | | | | | | | | | | | | 2 | 0.0029 |
| | | *1f | | | | | | | | | | | | 2 | 0.0029 |
| | | *1g | | | | | | | | | | | | 2 | 0.0029 |
| | | *1h | | | | | | | | | | | | 1 | 0.0015 |
| | | *1i | | | | | | | | | | | | 1 | 0.0015 |
| | *768K | *768K | | | | | | | | | | | | 19 | 0.028 |
| | *893S | *893S | | | | | | | | | | | | 2 | 0.0029 |

**Fig. 3** Block haplotypes in *DPYD* of block 1 (**a**), block 2 (**b**), block 3 (**c**), block 4 (**d**), block 5 (**e**), and block 6 (**f**) in a Japanese population. The nucleotide positions were numbered based on the cDNA sequence (A of the translational start codon is +1) or from the nearest exon. *White cell* wild-type, *gray cell* nucleotide alteration. §The haplotypes were inferred in only one patient and ambiguous except for marker SNPs

**Table 3** Linkages of haplotype-tagging SNPs with HapMap SNPs for *DPYD*

| Haplotype-tagging SNPs in *DPYD* | dbSNP ID (NCBI) | Block haplotype in this paper | HapMap SNPs with close linkages ($r^2 > 0.8$)[a] |
|---|---|---|---|
| 85T>C (Cys29Arg) | rs1801265 | Block 1 #*9* | rs10747488, rs7526108, rs4421623, rs4379706, rs4523551,rs11165921, rs9661794, rs6677116, rs6604093, rs17379561, rs10747491, rs10747492, rs12062845, rs7524038, rs10875112, rs4394693, rs10875113, rs4970722, rs9727548, rs10875118, rs9662719, rs12077442, rs4394694, rs9727976, rs4246515, rs6692580 |
| 496A>G (Met166Val) | rs2297595 | Block 1 #*166V* | rs2786543, rs2811215, rs2811214, rs2786544, rs2248658, rs11165897, rs2786490, rs2811203, rs2811202, rs2811200, rs2811198, rs2786503, rs2811196, rs2786505, rs2811195, rs2811194, rs12073839, rs6663670, rs7512910, rs2151563, rs2786509, rs3790387, rs3790389 |
| 1627A>G (Ile543Val) | rs1801159 | Block 2 #*5* | rs1415682, rs952501, rs2811187, rs2786778, rs2786774, rs2811183, rs17116806, rs2786780, rs1801159, rs2786771, rs2297780, rs2297779, rs12729863 |
| 1896T>C (Phe632Phe) | rs7556439 | Block 3 #*1b* | rs12073650 |
| IVS16-94G>T | rs7556439 | Block 5 #*1b* | rs693680, rs827500, rs499009, rs7518848, rs553388, rs507170, rs628959, rs991544, rs526645, rs1609519 |
| IVS18-39G>A | rs12137711 | Block 6 #*1b* | rs12120068, rs12116905 |

[a] All SNPs are in the same block

Taken together, our data demonstrated considerable differences in the haplotype distributions in blocks 1, 3 and 6 between Japanese and Caucasians.

## Discussion

This study provides Japanese data on the genetic variations of *DPYD*, a gene encoding a key enzyme catalyzing degradation of the well-known anticancer drug 5-FU. Nine novel (Ala10Glu, Tyr109Asn, Asn151Asp, Ile245Phe, Glu265Lys, Val515Ile, Phe524Leu, Ser556Arg, and Asn893Ser) and seven known nonsynonymous variations (Cys29Arg, Met166Val, Val335Leu, Ile543Val, Arg592Trp, Val732Ile, and Thr768Lys) were found in our Japanese population (Table 2 and Fig. 1). The association analysis between the genotypes and 5-FU pharmacodynamics is now on-going.

Uneven distributions of coding SNPs over 23 *DPYD* exons were pointed out in the previous review by van Kuilenburg (2004). The author indicated that 81% of all reported variations were confined to exons 2–14, representing 61% of the coding sequences, and typical hotspots of variation were localized in exons 2, 6, and 13. Our Japanese data also revealed that 17 out of 21 coding variations (81%) were localized in exons 1–14, and that more than three variations were detected in exons 5, 13, and 14 (Fig. 1). Recently, Hormozian et al. (2007) have reported that the common chromosomal fragile site on 1p21.2, *FRA1E*, spans 370 kb of genomic sequence between introns 8 and 18 of *DPYD*, and that its core region with the highest fragility is located between introns 12 and 16. The instability at the core of *FRA1E* might be associated with the high mutational rates and recombinogenic nature from intron 12 to 14 of *DPYD* (Fig. 1).

To estimate potential functional consequences of the amino acid substitutions, we examined whether the positions of amino acid changes are located in highly conserved areas or potentially critical regions of the molecule (for example, substrate recognition sites or binding regions of prosthetic groups). We also considered the locations of the residues in a three-dimensional (3D) framework provided by the crystal structures of pig DPD, which have recently been determined in complexes with NADPH and substrate (5-FU) (Dobritzsch et al. 2001) or inhibitors (Dobritzsch et al. 2002). The amino acid sequences of pig and human DPD are 93% identical (Mattison et al. 2002), and the substituted residues and their neighboring residues are conserved between both enzymes. From these points of view, it is speculated that at least two substitutions (Glu265Lys and Arg592Trp) might impact the structure and function of DPD as discussed below.

Glu265 is located on the loop following to the third $\beta$ sheet (II$\beta$3) in the FAD binding domain II (Dobritzsch et al. 2001). Glu265 is conserved among four mammalian species (human, mouse, rat, and pig), although it is replaced with aspartic acid in bovine and *Drosophila melanogaster* DPDs (Mattison et al. 2002). In the 3D structure of pig DPD (Fig. 5a), Glu265 is in close proximity to Lys259. The substitution, Lys259Glu, was