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特集：プリオン病と遅発性ウイルス感染症

孤発性プリオン病
（孤発性古典型CJD，視床型CJD，MM2皮質型CJD）

佐藤克也　　調　　漸　　江口勝美

孤発性プリオン病
(孤発性古典型CJD, 視床型CJD, MM2皮質型CJD)

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Clinical typing and diagnosis of sporadic human prion diseases
(classical sporadic CJD, MM2-cortical form CJD, MM2-thalamic form CJD)

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Abstract

We described recent knowledge and outline about a diagnosis and clinical typing of sporadic prion disease. Diagnostic procedure and classification based on a pattern of Western blotting of PrP^{Sc}, neuropathologic findings, and clinical features.

In addition, we described a clinical significance of total tau protein, significance of a diffusion-weighted images of MRI. Analysis of 112 cases of sporadic prion diseases showed 92.3% at positive rate. Sensitivity and specificity were 95.5% and 95.7%, respectively by total tau protein assay of 44 cases of prion diseases.

Key words: prion disease, diagnosis, clinical typing

はじめに

ヒトでのプリオン病の発症は人口100万人当たり1人とされており，比較的まれな疾患で，孤発性プリオン病，家族性プリオン病，感染性プリオン病の3つに分類できる(**表1**)．そのうち頻度の高いものは孤発性クロイツフェルト・ヤコブ病(CJD)であり，ヒトプリオン病の約80-85%を占めており，本稿では孤発性プリオン病について概説する．

孤発性プリオン病は大きく臨床経過にて2つに分類可能であり，急速進行型のCJDと比較的緩徐に進行するCJDに区別される．急速進行性のCJDは痴呆，不随意運動(ミオクローヌス),

表1　プリオン病の分類

孤発性
・孤発性クロイツフェルト・ヤコブ病 (sporadic Creutzfeldt-Jakob disease: sCJD)
・孤発性致死性不眠症(sporadic fatal insomnia: SFI)
家族性
・家族性クロイツフェルト・ヤコブ病
・ゲルストマン・ストロイスラー・シャインカー症候群 (Gerstmann-Sträussler-Scheinker syndrome: GSS)
・致死性家族性不眠症(fatal familial insomnia: FFI)
感染性
・医原性クロイツフェルト・ヤコブ病
ヒト成長ホルモン使用によるCJD
硬膜移植後CJD
・変異型クロイツフェルト・ヤコブ病(variant CJD)
・クールー病(Kuru)

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錐体路・錐体外路症状が急速に進行し3-7カ月で無動性無言に至る．いわゆる古典的CJDと呼ばれているものである．一方，緩徐に進行するCJDは慢性に経過し，痴呆症状・精神症状を主体とし，発症から1年以上経ってミオクローヌスや無動性無言になることを特徴とする一群がある．

現在CJDの分類は更に異常プリオン蛋白の分子量の違い・臨床症状・病理像にて大きく異なっていることから，一般的にParchi分類[1]が利用されているが，イギリスにおいてはCollinge分類[2]が利用される．

また孤発性CJDの病理学的特徴としては次の4点があげられる．①神経細胞の脱落・消失，②アストロサイトのグリオーシス，③海綿状変化，④抗プリオン染色による免疫染色が陽性となる．抗プリオン染色による免疫染色ではplaqueタイプとsynapticタイプの2種類に分類されるが，perineuronal patternとperivascular patternなどもみられる．

1. 異常プリオン蛋白のタイピング

まず，脳組織から抽出されたプリオン蛋白をプロテアーゼ処理した場合にプロテアーゼ抵抗性を示す異常プリオン蛋白のウエスタンブロットで検出されるバンドのパターンについて論じたい．Parchi分類[1]やCollinge分類[2]では異常プリオン蛋白のタイピングが骨格をなすからである．

異常プリオン蛋白は感染因子の本態と考えられており，正常プリオン蛋白と相互作用を起こし異常化を誘発する能力をもつために感染性を有するとされている．正常プリオン蛋白は脳組織から抽出されたプリオン蛋白をproteinase Kで処理すると完全に分解されるが，異常プリオン蛋白においてはペプチド鎖アミノ酸末端側の一部分が切り取られ，部分分解産物PrP27-30として残る．この残った部分分解産物つまり異常プリオン蛋白のウエスタンブロットのパターンによってタイピングする．

Parchiら[1]は異常プリオン蛋白のウエスタンブロットでのバンドを21kD: type1，19kD: type2の2つのタイプに分類した(図1)．Collinge[2]らは当初，異常プリオン蛋白のウエスタンブロットでのバンドを6つのタイプに分類したが，現在はCollinge[3]らは異常プリオン蛋白のウエスタンブロットでのバンドを4つに分類している．これら2つの分類があるためにタイピングについては混乱を来しやすいが，現状ではParchiらの異常プリオン蛋白のタイピングが汎用されている．その理由は異常プリオン蛋白がproteinase Kにてtype1とtype2に分かれる切断部位を明確にシークエンスしている点にある．Parchiら[4]のデータによると異常プリオン蛋白のproteinase KのN末端の切断部位がtype1ではコドン82が主体であり同一症例でもコドン78，84，86が混在することが多いが，type2ではコドン97が主体であり，コドン92，99，103が混在することを示した．一方，異常プリオン蛋白のproteinase KのC末端の切断部位がコドン228-230のことが多い(図2)．

我が国における異常プリオン蛋白のタイピングは著者ら[5,6]が孤発性・家族性・感染性を含め11タイプ計34症例での検討を報告した．

ところで最近自験例でもCJD患者一個人でもbrain内にtype1とtype2とがよく混在するケースを経験する．混在するケースは今まであまり強調されていないが，近年，実際には混在する例がほとんどではないかという解釈が主流になりつつある．つまり異常プリオン蛋白のタイピングは単にウエスタンブロット法のみでなく，詳細な臨床症状と病理(HE染色・免疫染色)をよく観察する必要がある．

2. プリオン蛋白の正常多型

プリオン蛋白遺伝子は第20染色体短腕上にある．その遺伝子は253個のアミノ酸をコードし，現在まで30個の遺伝子異常が報告されている．正常多型は現在コドン129，171，219の3カ所で報告されている．そのなかでもコドン129が臨床症状・病理像に影響を受けるが，コドン219がプリオン病のなりやすさなどを反映している．

コドン129の正常多型では我が国において[7]

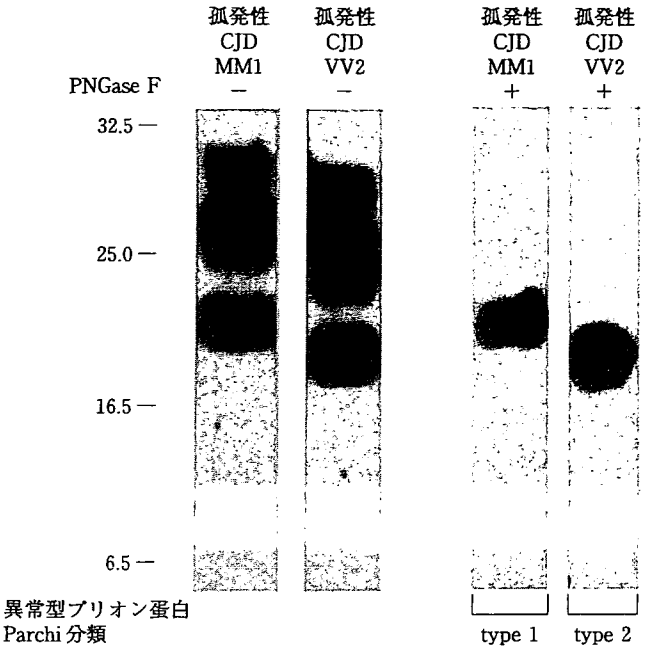

図1 異常プリオン蛋白のタイピング

異常プリオン蛋白のウエスタンブロットにおいて2つの異常プリオン蛋白のタイプつまり蛋白量の違いを示している．元々糖鎖部位が2カ所あり，3本のバンドを形成し，PNGaseF処理を行うと糖鎖除去酵素に1本バンドになる．

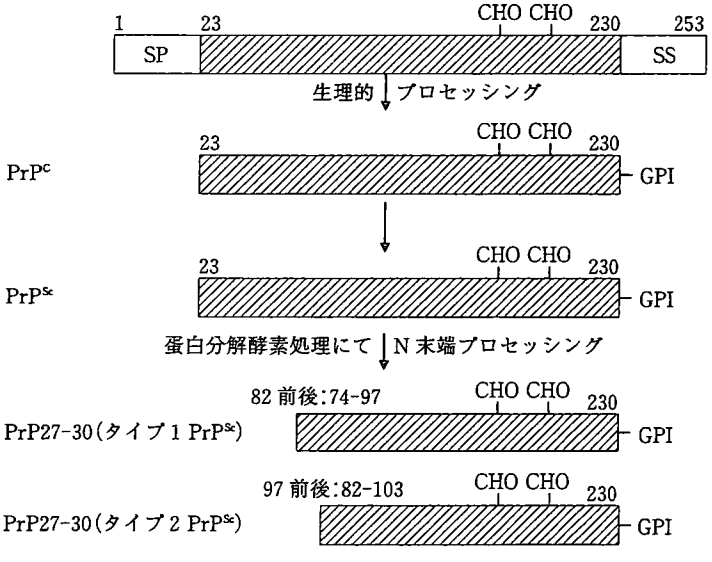

図2 異常プリオン蛋白のタイピングのプロセッシング(文献[6]より引用)

コドン129のメチオニン(M)とバリン(V)の多型ではMMが90.9%，MVが9.0%，VVが0.1%となっている．一方，コドン219の多型[8]はEEが88%，EKが12%，KKが0.01%である．この正常多型の頻度は人種間により非常に異なる．

表2 プリオン蛋白遺伝子コドン129番多型と異常プリオン蛋白型による孤発性クロイツフェルト・ヤコブ病のサブグループ

遺伝子型・蛋白型	MM1	MM2	MV1	MV2	VV1	VV2
病型	典型的CJD	皮質型/視床型	典型的CJD	失調型	痴呆型	失調型
プリオン蛋白沈着パターン	シナプス型	シナプス型	シナプス型	シナプス型 プラーク型	シナプス型	シナプス型 プラーク型
ミオクローヌス	+	−	+	+	−	+
周期性同期性放電	+	−	+	まれ	−	まれ
14-3-3蛋白	+	+	+	まれ	+	+
発症年齢	60歳代	60歳代	60歳代	60歳代	20歳代	60歳代
進行速度	亜急性	緩徐	亜急性	緩徐	緩徐	亜急性

コドン219は金子ら[9]が提唱したprotein Xがバインディングする最も重要な部位と考えられており，モデルマウス系においてもコドン219はグルタミン酸(E)からリシン(K)に変換しているマウスでの発症は認められていない．いまだにコドン219のリシンをホモでもっているケースではCJDの発症したケースは報告されていない．

3. ヒト孤発性CJDのParchi分類

一般的にParchi分類[1]が利用されているので，これについて述べる．プリオン蛋白のコドン129の多型に加えて，PrP^{Sc}のウエスタンブロットでのバンドのパターン・臨床症状・病理像に従い6つに分類されている(表2)．

a. MM1またはMV1

MM1またはMV1は古典型CJDの臨床症状は最も一般的によくみられるものは孤発性のCJDである．前駆症状として食欲不振，頭痛，全身倦怠感，睡眠障害がみられることがあり，初期症状としては進行性の痴呆症状に加えて，精神障害，高次脳機能異常，運動失調，めまい感などがあげられる．発症から数カ月以内に高度の痴呆となり，会話も不能となり自発言語も乏しくなる．起立・歩行が不能となり，次第に無動性無言へと陥る．ほとんどは発症から約6カ月-1年ほどで死に至る．

b. VV2

MM1・MV1に次いでヨーロッパでは最も多い形になる．失調症状での発症を特徴とする．平均罹病期間[10]は6カ月ほどで初期に認知機能障害や精神症状に加え眼球運動障害が認められる．後期になりミオクローヌス発作は観察されるが，脳波上PSDは目立たず，髄液中の14-3-3蛋白は80%に陽性であった．病理学的に海綿状変化，アストロサイトのグリオーシス，神経脱落の3徴がみられる．

c. MV2

表現型はMV1に類似し，失調，進行性痴呆を呈する．VV2に比べ認知機能障害や精神症状が強く，認知機能障害や精神症状が前景に出やすい．病理像はKuru斑の出現が特徴的である．罹病期間は平均17カ月とやや長い．錐体外路症状を呈しやすく，多系統萎縮症やAlzheimer病に間違えられやすい傾向にある．

d. MM2皮質型

60歳以降に認知症症状にて発症するが，初期はAlzheimer病に類似している．Parchi[1]が報告したときにミオクローヌスは認められないと報告したのだが，実際[11,12]にはミオクローヌスは認められることが多く，しかも病中期ごろに認めやすい．中期では錐体外路症状，ミオクローヌスを認め，後期で小脳失調を認める．罹病期間は16カ月程度である．教科書的にはこの様だが経験側的には初期に高次機能障害，特に保続や失行を認めるケースが多い．また診断には拡散強調画像において皮質での高信号領域が認められる．髄液検査[13]では14-3-3蛋白と比較して総タウ蛋白やS-100b蛋白が高値を示すことが多い．Krasnianskiら[14]が12例を報告し

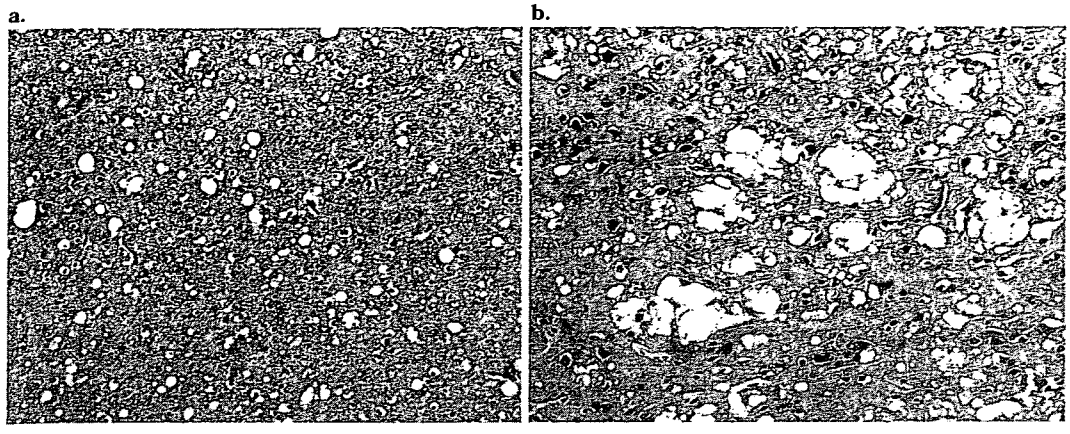

図3 古典的CJDとMM2-皮質型の海綿状変化
CJD患者での大脳皮質の海綿状変化であり，a: MM1，b: MM2-皮質型であり，等倍での観察においても海綿状変化はaに比べbの方が粗造で空胞変性がbの方が大きい．

ているがMM2皮質型で最も他の病型と異なるのは大きな空胞形成(海綿状変化)である(図3)．

e. MM2視床型

臨床上，診断が最も難しい病型である．元来，MM2視床型は'視床型CJD'あるいは'視床変性症'などと診断されていた症例に該当するものであり，最近Parchi[15]は'孤発性致死性不眠症'と報告している．罹病期間は平均24カ月で失調，視覚異常，認知機能障害が特徴的である．進行すると不眠症，認知症症状，運動障害を呈する．髄液検査の14-3-3蛋白がほとんどの症例で陰性である．病理像でも視床のみに限局し，グリオーシスや神経細胞脱落が認められる．

f. VV1

報告例が少なく平均発症年齢が39歳で罹病期間は平均15カ月である．症状はFTDに類似した症状を呈する脳波は徐波傾向を示すが，PSDは示さない．Meissnerらの報告[16]の9例でのまとめでは平均発症年齢は44歳，罹病期間は平均21カ月．MRI上は特徴的な所見は認められないが，FLAIRやT2信号での大脳皮質において高信号領域を示すことは多い．髄液所見では14-3-3蛋白は全例陽性だが，他の総タウ蛋白やS-100b蛋白の検出率は低いと考えられている．

4. 検査所見(脳波，MRI，髄液)

従来は補助的診断として通常の頭部CTやMRIに加えて比較的特異度の高い検査としては脳波検査しか行われていなかった．脳波では実際には病初期では，周期性の放電は明らかではなく高振幅徐波が認められ，次いで徐々に特徴的な周期性同期性放電が観察され，最後には低振幅徐波となり周期性同期性放電も観察されなくなることが明らかになってきており，脳波異常だけでは病初期の補助診断としては不十分と考えられている．

補助検査に頭部MRI拡散強調画像と髄液生化学検査がある．頭部MRIでは病状初期から急性期脳梗塞巣の検出に頻用される頭部MRIの拡散強調画像で大脳皮質に沿った高信号や大脳基底核領域の高信号が認められる．MRI拡散強調画像の異常高信号はCJDの早期診断法としては極めて有用である．

a. MRI拡散強調画像

MRIでの異常信号は孤発性CJDにおいて当初T2で両側対称性の線条体の高信号が報告され，更にFLAIR画像において様々に報告されている．1997年Demaerelら[17]は拡散強調画像での意義を報告し，注目を浴びた．Youngら[18]は40症例において拡散強調画像で感度91%，特異度95%があり，FLAIRで感度・特異度が94

表3 孤発性クロイツフェルト・ヤコブ病の診断基準

I. 従来から用いられている診断基準(Mastersら，1979ほか)
A. 確実例(definite)
特徴的な病理所見，またはWestern blotや免疫染色法で脳に異常プリオン蛋白を検出.
B. ほぼ確実例(probable)
1. 急速進行性認知症.
2. 次の4項目中2項目以上を満たす.
a. ミオクローヌス
b. 視覚または小脳症状
c. 錐体路または錐体外路徴候
d. 無動性無言
3. 脳波上で周期性同期性放電(PSD)
C. 疑い例(possible)
上記のBの1および2を満たすが，脳波上PSDがない場合.
II. 拡大診断基準(WHO 1998)
上記の診断基準のCの疑い例possibleに入る例で，脳波上PSDがなくても，脳脊髄液中に14-3-3′蛋白が検出され臨床経過が2年未満の場合，ほぼ確実例probableとする.
ルーチン検査で，CJDにかわる他の診断が除外されることが必要.

%と報告した．Tschampaら[19]はMRIにて尾状核・被核を含めた基底核において典型的な所見を示し，検討し，442症例中(T2画像は184症例，FLAIR画像は132症例，拡散強調画像は75症例)59.7%であったとしている．このデータの解離の原因はMRI拡散強調画像の撮像条件の標準化と異常信号に対する解釈の難しさにあると思われる．今後の我が国でもMRI拡散強調画像の標準化と異常信号に対するCJDと診断する際の特異度を上げる方法が重要になってきている．MRI先進国である我が国ではShigaら[20]が報告しているが，26症例中感度92.3%，特異度93.8%であった．著者らも44症例における検討を行ったが，感度90.9%，特異度97.8%を示していた(**図4**，**表4**).

b. 髄液検査

WHOのCJDの診断基準[21]には14-3-3蛋白が補助的診断基準の一つとして加えられている(**表3**)．我が国における孤発性CJDの脳脊髄液検査について著者ら[22]は14-3-3蛋白についての有用性について報告したが，NSEおよび総タウ蛋白については現在まで詳細な報告はない．

近年，Ottoら[23]がヨーロッパの孤発性CJD患者約300例について14-3-3蛋白，NSE，総タウ蛋白の感度について検討を行い，総タウ蛋白が最も感度が高く，特異度も高いことを報告し，次いで著者ら[22]も我が国での脳脊髄液検査の14-3-3蛋白，NSE，総タウ蛋白の感度・特異度について報告した.

1) 脳脊髄液中の14-3-3蛋白について

CJD患者での脳脊髄液中の14-3-3蛋白の最初の報告は1996年Hsichら[24]がCJD患者の脳組織と正常人の脳組織を二次元電気泳動で比較，検討し，14-3-3蛋白を同定しCJD患者での脳脊髄液での特異性を示した．同年Zerrら[25]がCJD患者の脳脊髄液をHsichと同様な方法で解析し14-3-3蛋白を同定し，脳脊髄液中での14-3-3蛋白の有用性を示した.

我が国における大規模での研究データは示されていない．平成16年度の厚生労働省の厚生科学研究班会議での袖山らの報告[26]では14-3-3蛋白の検出はdefinite caseで74%，probable caseで92%，possible caseで86%であった．自験例としてCJD患者44例について14-3-3蛋白を施行し，29/33(88.6%)の感度を示している(**図4**，**表4**).

14-3-3蛋白の検出は脳脊髄液の採取の時期によってもCJDのtypeによってもかなり感度・特異度が異なる．脳脊髄液の採取の時期においてはOttoら[23]やVan Everbroeckら[27]の同一患者

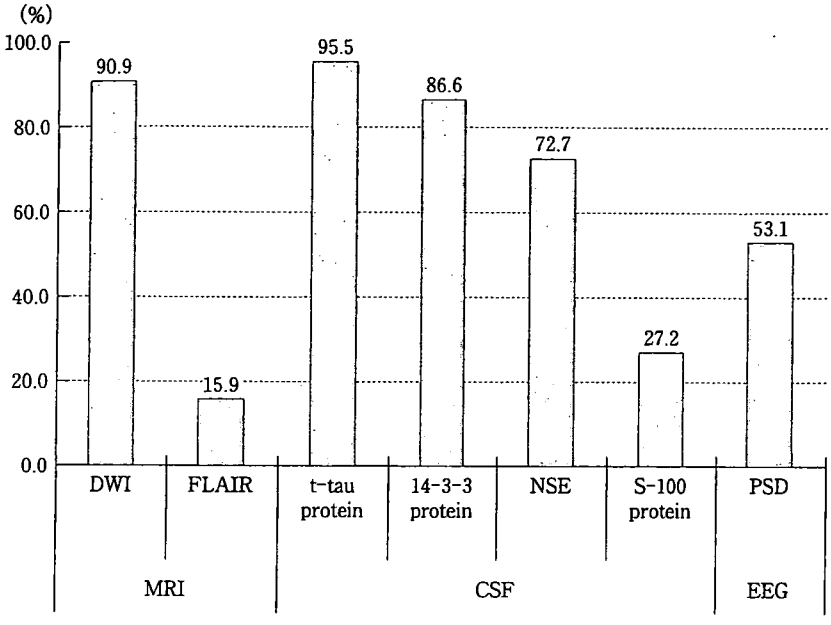

図4 髄液検査とMRI検査での検討
n=44

表4 CJD患者44例における髄液とDWIとの組み合わせによる感度・特異度

	TP	TN	FP	FN	感度	特異度
total tau protein	42	88	4	2	95.5%	95.7%
14-3-3 protein	39	88	4	5	88.6%	95.7%
total tau protein and 14-3-3 protein	39	88	4	5	88.6%	95.7%
MR-DWI	40	90	2	4	90.9%	97.8%
MR-DWI and total tau protein	38	90	2	6	86.4%	97.8%
MR-DWI and 14-3-3 protein	38	90	2	6	86.4%	97.8%

TP: 真陽性, TN: 真陰性, FP: 疑陽性, FN: 偽陰性.

でのステージの違いではないものの，発症からの時期において患者の検出率を検討しており，初期の段階での検出感度が低いとされている．また発症してから1年以上経つと感度70%以下と再び低下しており，このような病期では診断上注意を要する．発症後早期に検査できた自験例では一度14-3-3蛋白を検出できなかったケースの患者においても2週間-1カ月程度経つと検出できた例を数例経験しており，病状の進行に伴い14-3-3蛋白の検出率は変化する可能性を含んでいる．一方，CJDの病型では大きく分け，感染性，家族性，孤発性の3つに分類されることは周知のとおりであるが，孤発性CJDのParchi分類に基づいて14-3-3蛋白の解析を行った報告では14-3-3蛋白の検出率はtype1(129MM 92%，129MV 100%，129VV 100%)，type2(129MM 75%，129MV 50%，129VV 83%)と明らかにtype2において低い．症例が少ないので明確ではないが，MM type2-thalamic formでの検出率が非常に低いものと思われる．type2と推定される症例では脳脊髄液の14-3-3蛋白の検出率が低いことを念頭に

置いて診断を進めなければならない.

2) 脳脊髄液中の総タウ蛋白について

1997年Ottoら[23]は脳脊髄液中の総タウ蛋白が高いことを報告し，更に2002年に[23]297人のCJD患者(definite case 109人，probable case 55人，possible case 39人；others case 85人，iatrogenic case 1人，genetic case 8人)の脳脊髄液における14-3-3蛋白と総タウ蛋白の比較を行った．これらの報告では総タウ蛋白が感度94%，特異度90%TP報告されており，14-3-3蛋白より特異度・感度において優れていることを確認した．著者ら[22]は我が国の患者で検討し神経疾患でCJD患者13例とnon-CJD患者87例計100例を対象としOttoらと同様にROCカーブにてcut-off 1,260 pg/mlとし感度92.3%，特異度97%を示した．現在CJD患者44例を対象としても感度97%，特異度97%となる(**図5**)．この症例の検討において偽陽性を示した症例はAlzheimer型痴呆と脳血管性痴呆であったが，Ottoら[23]の検討において偽陽性を示したケースはAlzheimer型痴呆，脳血管性痴呆，アルコール脳症，悪性リンパ腫であった．そこで著者ら[22]はAlzheimer型痴呆と脳血管性痴呆とを区別することを目的としてリン酸化タウ蛋白と総タウ蛋白との比をとり，特にAlzheimer型痴呆とを区別することは可能であることを報告した(**図5**)．14-3-3蛋白と総タウ蛋白を比較して特異度・感度において総タウ蛋白が優れており，14-3-3蛋白の欠点と思われるものを改善している．今後総タウ蛋白は14-3-3蛋白に代わり脳脊髄液の検査の主役を占めると考えられる．最後に最近症例の蓄積において多数例の症例において病型分類と髄液マーカー，画像マーカーとの検討を行った．112症例での検討結果を示す(**表5，6，図6**)．

おわりに

現在，CJDの治療薬の開発およびCJD患者に対する投与が始まったばかりではあるが，今後，正確に早期診断を行うことが非常に重要な意味をもつ．現在の厚生労働省の診断基準やWHOの診断基準では早期診断を行うための画像検査や髄液検査の補助的な検査の意義が明らかになる以前のものであり，明らかに時代遅れになっている．特に欧米に比しても我が国はMRI拡散強調画像の先進国であるという利点を生かし，多数施設でのMRI拡散強調画像の検討を行うとともに，髄液生化学マーカーとの有用性の比較検討，併用の臨床的意義を明らかにする必要があると考える．

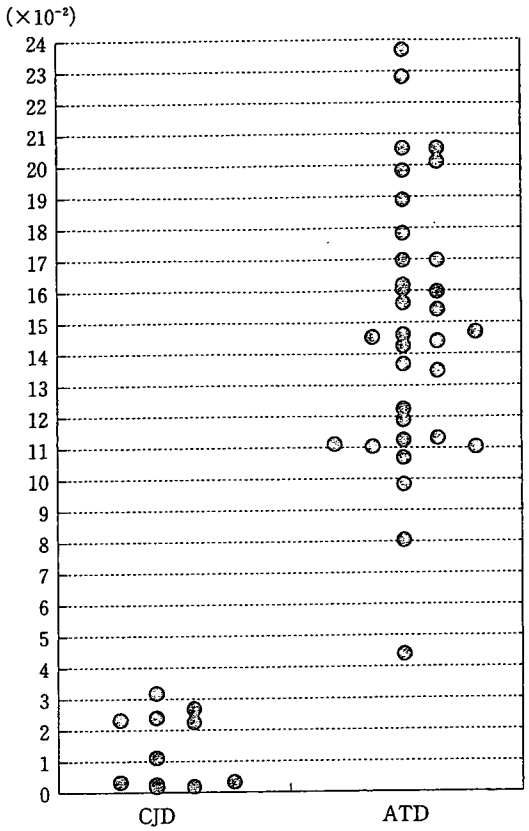

図5 髄液中のリン酸化タウ蛋白と総タウ蛋白の比

表5 112例の内訳

・古典型CJD	94例
・MV非典型例	1例
・MM2皮質型	4例
・家族性 180	5例
144塩基対挿入	2例
・硬膜移植後CJD	3例
・GSS 102	3例

表6 112例の髄液検査・画像検査の陽性率

	総数	14-3-3蛋白	総タウ蛋白	DWI
古典型CJD	94例	95.7%	98.9%	91.1%
MV非典型	1例	0.0%	0.0%	100.0%
MM2皮質型	4例	50.0%	100.0%	100.0%
家族性				
180	5例	40.0%	80.0%	100.0%
144塩基対挿入	2例	0.0%	50.0%	不明
硬膜移植後CJD	3例	100.0%	100.0%	100.0%
GSS	3例	0.0%	33.3%	不明
	112例	86.6%	95.5%	92.3%

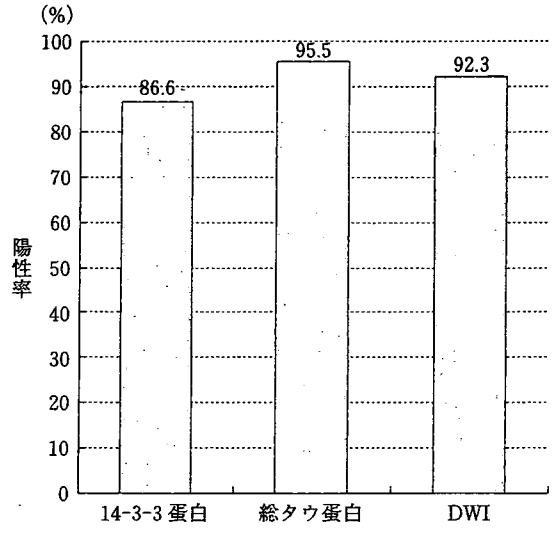

図6 髄液検査とMRI検査での検討
n=112

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REVIEW

Molecular biology of prion protein and its first homologous protein

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Abstract : Conformational conversion of the normal cellular isoform of prion protein, PrP^C, a glycoprotein anchored to the cell membrane by a glycosylphosphatidylinositol moiety, into the abnormally folded, amyloidogenic prion protein, PrP^{Sc}, plays a pivotal role in the pathogenesis of prion diseases. It has been suggested that PrP^C might be functionally disturbed by constitutive conversion to PrP^{Sc} due to either the resulting depletion of PrP^C or the dominant negative effects of PrP^{Sc} on PrP^C or both. Consistent with this, we and others showed that mice devoid of PrP^C (PrP-/-) spontaneously developed abnormal phenotypes very similar to the neurological abnormalities of prion diseases, supporting the concept that functional loss of PrP^C might at least be partly involved in the pathogenesis of the diseases. However, no neuronal cell death could be detected in PrP-/- mice, indicating that the functional loss of PrP^C alone might not be enough to induce neuronal cell death, one of major pathological hallmarks of prion diseases. Interestingly, it was recently shown that the first identified PrP-like protein, termed PrPLP/Doppel (Dpl), is neurotoxic in the absence of PrP^C, causing Purkinje cell degeneration in the cerebellum of mice. Although it is not understood if PrP^{Sc} could have a neurotoxic potential similar to PrPLP/Dpl, it is very interesting to speculate that accumulation of PrP^{Sc} and the functional disturbance of PrP^C, both of which are caused by constitutive conversion, might be required for the neurodegeneration in prion diseases. J. Med. Invest. 54 : 211-223, August, 2007

Keywords **:** *prion, prion protein, prion protein-like protein, knockout mice, neurodegeneration*

INTRODUCTION

The normal cellular isoform of prion protein, designated PrP^C, is a membrane glycoprotein abundantly expressed in the central nervous system (CNS), particularly in neurons (1). Its structural counterpart, the abnormally folded, amyloidogenic isoform, termed PrP^{Sc}, is specifically present in the tissues affected by prion diseases (1). Prion diseases are a group of fatal neurodegenerative disorders including Creutzfeldt-Jakob disease in humans and scrapie and bovine spongiform encephalopathy in animals (2). The causative agents of the diseases, the so-called prions, are very different from conventional pathogens, such as bacteria and viruses (3). Prions lack a nucleic acid genome (3). According to the widely accepted protein-only hypothesis, prions are assumed to consist of PrP^{Sc} alone (4). However, the exact nature of prions still remains controversial. Here, I will discuss the nature of prions and the roles of PrP in the pathogenesis of prion diseases.

I will also discuss the normal functions of PrP^C and its antagonistic function to the first identified PrP-like protein, termed PrPLP/doppel (Dpl) (5).

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PrPLP/Dpl is neurotoxic when ectopically expressed in neurons, causing Purkinje cell degeneration in mice, and PrP^C antagonizes the neurotoxicity, rescuing the mice from neurodegeneration (6, 7). However, unlike PrP^C, PrPLP/Dpl seems to have no potential to convert to a PrP^{Sc}-like infectious isoform (6, 8).

NORMAL AND ABNORMAL ISOFORMS OF PRP

Normal isoform of PrP

The gene for PrP, designated *Prnp*, is located on chromosomes 2 and 20 in human and mouse, respectively (9). Human and hamster *Prnp* consists of two exons (9). On the other hand, mouse, sheep, and rat *Prnp* contain three exons with exon 3 analogous to exon 2 of human and hamster *Prnp*. The protein coding sequence is present in the last single exon in all mammals. *Prnp* is constitutively expressed in various tissues, with highest expression in brain, particularly in neurons, and, to a lesser extent, in others including spleen, kidney, lung, and heart (10).

Mouse *Prnp* encodes the precursor protein consisting of 254 amino acids (Fig. 1). The 22 N-terminal hydrophobic amino acids are removed as a signal peptide when the nascent precursor protein enters the endoplasmic reticulum (ER) (Fig. 1). The 23 C-terminal hydrophobic amino acids are also cleaved as a glycosylphosphatidylinositol (GPI) -anchor signal sequence in the ER and, at the same time, a GPI anchor moiety is attached to the C-terminus of the serine residue at position 231 (Fig. 1) (1). Then, the protein is transported to the Golgi apparatus, where two asparagine residues at positions 181 and 197 undergo N-glycosylation (Fig. 1), and finally to the cell surface along the secretory pathway. As a result, mature PrP^C, consisting of the amino acid residues 23-231, is expressed as a membrane glycoprotein anchored to the cell surface via a GPI moiety.

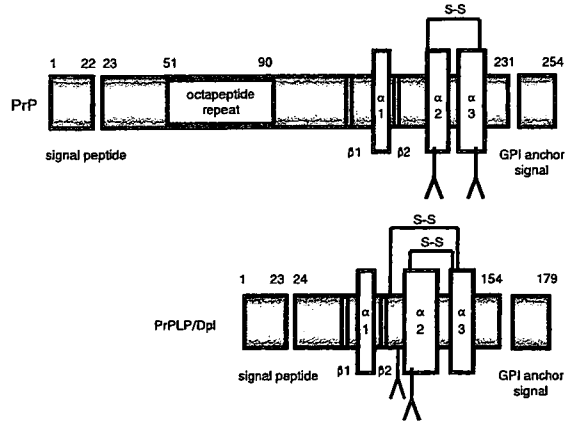

Fig. 1. Schematic structures of PrP and PrPLP/Dpl. α and β indicate α-helix and β-strand, respectively. S-S and Y indicate a disulfide bond and N-glycosylation, respectively. Arabic figures represent amino acid positions.

Abnormal isoform of PrP

The abnormal isoform, PrP^{Sc}, is specifically produced within prion infected cells, particularly in neurons. We and others showed that PrP^{Sc} failed to be produced in mice devoid of PrP^C (PrP-/-) (11-14), indicating that PrP^C is essential for the generation of PrP^{Sc}. Moreover, it was reported that PrP^{Sc}-like PrP could be generated from PrP^C in certain conditions *in vitro* (15). Therefore, it is thought that PrP^{Sc} is produced from PrP^C. However, it remains unknown whether or not PrP^{Sc} is produced from either mature PrP^C or immature unfolded PrP, or both.

PrP^{Sc} is identical to PrP^C in amino acid sequence. However, structural analysis of PrP^C and PrP^{Sc} using circular dichroism revealed marked differences in the protein structures of both proteins (Table 1). PrP^C has a lower content of β-sheet strands (3%) but a higher α-helix content (42%) (16). In contrast, PrP^{Sc} has a higher β-sheet content (43%) (16). It is therefore postulated that the transition of α-helices into β-sheet strands within PrP might be a key step in the generation of PrP^{Sc}.

Biochemical and structural properties of PrPs

PrP^C and PrP^{Sc} possess markedly different biochemical properties from each other, particularly in detergent solubility and resistance to proteinase digestion, probably due to the structural differences. PrP^C is highly soluble and easily digested by proteinase K whereas PrP^{Sc} readily aggregates to form

Table 1 Different biochemical and structural properties of PrP isoforms

PrP isoforms	secondary structure content		detergent solubility	proteinase K digestion
	α-helix	β-sheet		
Normal isoform (PrP^C)	42%	3%	soluble	sensitive
Abnormal isoform (PrP^{Sc})	30%	43%	insoluble	relatively resistant

amyloid fibers and is relatively resistant to the digestion (Table 1) (1). These different biochemical properties are useful to distinguish PrP^{Sc} from PrP^{C}.

PrP^{Sc} remains structurally unresolved due to its predisposition to form aggregates. In contrast, the protein structure of PrP^{C} was resolved by nuclear magnetic resonance (NMR) analysis. According to NMR analysis, the N-terminal domain of PrP^{C} is highly flexible and lacks identifiable secondary structure while the C-terminal domain forms a globular structure with three α-helices and two short antiparallel β-strands (Fig. 1) (17). The second and third helices are linked by a disulfide bond (Fig. 1) (17).

The N-terminal domain includes a PrP-specific region, the so-called octapeptide repeat (OR) region, in which 8 amino acids are repeated 5 times in tandem (Fig. 1). This region is considered to bind Cu^{2+} via histidine residues and mediate anti-oxidative activities by activating Cu^{2+}-dependent antioxidant enzymes such as superoxide dismutase via transfer of bound Cu^{2+} to the enzymes (18, 19). However, the exact function of this region in anti-oxidative activities remains to be elucidated.

THE FIRST PRP-LIKE PROTEIN (PRPLP/DPL)

PrPLP/Dpl and PrP

We and others isolated a novel gene, termed *Prnd*, encoding the first PrP-like protein, PrPLP/Dpl, about 16-kb downstream of the PrP gene, *Prnp* (5, 7). PrPLP/Dpl is also a GPI-anchored membrane glycoprotein (5). Like PrP^{C}, PrPLP/Dpl is first translated into the precursor protein consisting of 179 amino acids and then undergoes several modifications, including cleavage of the 23 N-terminal and 25 C-terminal hydrophobic residues as a signal peptide and a GPI-anchor signal, respectively, N-glycosylation at two sites, and formation of two disulfide bonds (Fig. 1) (20).

PrPLP/Dpl and PrP share ~23% identical amino acids (5, 7). However, PrPLP/Dpl lacks a region corresponding to the N-terminal part of PrP^{C} (5, 7). The protein structural analysis clearly showed that PrPLP/Dpl is a structural homologue of the C-terminal globular domain of PrP^{C}, composed of three α-helices and two short antiparallel β-strands (Fig. 1) (21).

Normal functions of PrPLP/Dpl

PrPLP/Dpl mRNA is expressed in various tissues of adult wild-type mice, including the testis, heart, spleen and skeletal muscle (22). To investigate the physiological functions of PrPLP/Dpl in mice, Behrens, *et al.* produced mice devoid of PrPLP/Dpl, designated $Prnd^{neo/neo}$ mice (23). Interestingly, male mutant mice were sterile whereas female mutant mice were fertile. The testes in these mutant mice were macroscopically normal. However, the number of spermatozoa and motility of mutant sperm were significantly decreased. Moreover, the mutant sperm exhibited abnormal morphologies and impaired acrosome function. Consistently, it has been reported that PrPLP/Dpl is expressed in spermatids in mice and spermatozoa and Sertoli cells in humans (23, 24). These results indicate that PrPLP/Dpl is involved in spermatogenesis.

In contrast to PrP^{C}, PrPLP/Dpl was undetectable in the brains of adult wild-type mice (22). However, in neonatal mice, we found substantial expression of PrPLP/Dpl mRNA in their brains, preferentially in blood vessel endothelial cells. PrPLP/Dpl mRNA was already expressed 1 day after birth, peaked by around 1 week, and then decreased to an undetectable level by at least 8 weeks (22). Therefore, such developmental regulation of PrPLP/Dpl expression in brain blood vessels suggests that PrPLP/Dpl may be involved in the development of brain blood vessels and/or blood-brain barrier. However, no pathological abnormalities were detected in the tissues of $Prnd^{neo/neo}$ mice, including the brain, heart, spleen and skeletal muscle (23).

PRION RESEARCH IN MICE DEVOID OF PRP

Protein-only hypothesis and mice devoid of PrP

The protein-only hypothesis postulates that a prion is constituted of PrP^{Sc} alone (25). According to the hypothesis, a prion or PrP^{Sc} interacts with PrP^{C} expressed on the cell surface and induces changes in the conformation of the interacting PrP^{C} into that of PrP^{Sc}, resulting in generation of a new PrP^{Sc} molecule, or propagation of a prion (Fig. 2). However, the hypothesis is controversial. If the hypothesis is true, prions cannot propagate when PrP^{C} is absent. In contrast, if prions can propagate without PrP^{C}, the hypothesis is clearly negated. In other words, if PrP-/- mice can support prion propagation, the hypothesis is wrong, and vise versa.

To investigate validity of the protein-only hypothesis, we generated a line of PrP-/- mice, referred to

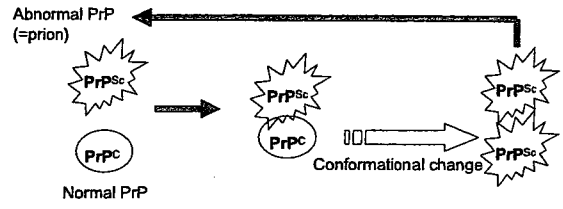

Fig. 2. A prion replication model according to the protein-only hypothesis. A prion is constituted of the abnormal isofrom of PrP, PrP^{Sc}, interacts with the normal isoform of PrP, PrP^{C}, and induces the conformational changes of the interacting PrP^{C} to produce a new molecule of PrP^{Sc} or a prion. The newly produced PrP^{Sc} or prion also converts another PrP^{C} into PrP^{Sc} or prion in the same way.

as Ngsk PrP-/- mice, and intracerebrally inoculated them with a mouse-adapted Fukuoka-1 prion (14). Wild-type (PrP+/+) mice developed disease-specific symptoms at 138±12 days and died 143±14 days after inoculation (Table 2). Microscopic examinations of the brains of these diseased mice showed profound vacuolation and gliosis, both of which are hallmarks of the pathological changes in prion diseases. In addition, PrP^{Sc} was markedly accumulated and prions were propagated in their brains. In contrast, no Ngsk PrP-/- mice showed such specific symptoms. They were all alive at least by 460 days after inoculation (Table 2). No disease-specific pathologies were observed in the brains of Ngsk PrP-/- mice sacrificed 400 days after inoculation. Moreover, neither accumulation of PrP^{Sc} nor prion propagation could be detected in their brains. Other investigators also showed similar results using other lines of PrP-/- mice (11-13). These results indicate that PrP^{C} is essentially required for prion propagation, and that prion propagation is linked to accumulation of PrP^{Sc}, strongly supporting the protein-only hypothesis.

Prolonged incubation times and less accumulation of PrP^{Sc} in mice heterozygous for PrP

We also inoculated Ngsk PrP+/- mice with the Fukuoka-1 prion (14). Compared with PrP+/+ mice, Ngsk PrP+/- mice developed the disease with considerably retarded incubation times of 259±27 days and died 269±27 days after inoculation (Table 2). The clinical symptoms and pathological changes in diseased Ngsk PrP+/- mice were indistinguishable from those of diseased PrP+/+ mice. However, very strangely, amounts of PrP^{Sc} accumulated in the brains of terminal PrP+/- mice were only half of those in terminal PrP+/+ mice (14). These results indicate that the expression levels of PrP^{C} prior to infection affect the timing of onset of disease and the accumulation levels of PrP^{Sc} but not the final severity and pathology of disease, and that PrP^{Sc} levels are not correlated with disease progression.

NORMAL FUNCTIONS OF PRP IN NEURONS AND GLIA

Higher brain functions and PrP

Since PrP^{C} is abundantly expressed in pyramidal neurons of the hippocampus, in which learning and memory processes are integrated, it has been suggested that PrP^{C} might be involved in learning and memory processes. Büeler, *et al.* produced a line of PrP-/- mice, Zrch I PrP-/-, and subjected them to behavioral tasks, such as a swimming navigation test and a Y-maze discrimination test (26). However, no different performance could be detected in these tests between the mutant mice and control PrP+/+ mice (26). On the other hand, Nishida, *et al.* reported poor performance in Ngsk PrP-/- mice using other behavioral tests, including a water-finding test and a conditioned passive-avoidance test (27). Thus, it may be possible that PrP^{C} is involved in certain types of learning and memory.

Collinge, *et al.* showed that long-term potentiation (LTP), a form of synaptic plasticity that is thought to be important for memory formation, was impaired in the hippocampal CA1 neurons of Zrch I PrP-/- mice using electrophysiological studies (28). Similar results were reported in another line of PrP-/- mice, Npu PrP-/- mice (29). Therefore, these results seem to support that PrP^{C} is involved in the processes of learning and memory. However, other investigators reported no such electrophysiological abnormalities in the hippocampus of Zrch I PrP-/- mice (30).

It was also shown that PrP^{C} is involved in the regulation of circadian rhythm (31). In both Zrch I PrP-/- and Npu PrP-/- mice, much more fragmented sleep was observed than in PrP+/+ mice (31). Moreover,

Table 2 Ngsk PrP-/- mice were resistant to prion disease.

Mouse genotype	Incubation time (mean±SD days)	Survival time (mean±SD days)
Wild-type	138±12	143±14
Ngsk PrP+/-	259±27	269±27
Ngsk PrP-/-	<460	<460

the mutant mice exhibited a much longer activity period of 23.9 h under constant darkness, compared to 23.3 h in PrP+/+ mice (31).

Axonal myelination and PrP

We found many vacuoles in the spinal cord and peripheral nervous system of Ngsk PrP-/- and Zrch I PrP-/- mice (32). Most of the vacuoles were surrounded by an enlarged myelin sheath, but in some cases splits within a myelin sheath formed vacuoles (32). In addition, large myelinated fibers were reduced in number and remaining axons were thinly myelinated (32). Subsequently, we could rescue Ngsk PrP-/- mice from the demyelination by transgenically expressing mouse PrP^C (32). These results indicate that PrP^C is involved in the organization of the myelin sheath.

PrP^C is expressed on the surface of oligodendrocytes and Schwann cells (33, 34), both of which form myelin sheaths in the CNS and the peripheral nervous system, respectively. It is therefore conceivable that PrP^C functions as an adhesion molecule within a myelin sheath and/or between a myelin sheath and an axon to form a tightly compacted myelin sheath. This might be consistent with the result that some vacuoles were formed due to splits within myelin sheaths. It is alternatively possible that PrP^C could be a trophic factor for these glial cells.

PRP AND PRPLP/DPL IN NEURODEGENERATION

Purkinje cell degeneration among different lines of mice devoid of PrP

No neuropathological abnormalities were reported in Zrch I PrP-/- and Npu PrP-/- mice (26, 35). However, very strangely, we noticed that Ngsk PrP-/- mice showed ataxic gait around 70 weeks after birth (36). In these ataxic mice, cerebellar Purkinje cells were dramatically decreased in number due to their degeneration and the molecular layer also became very thin, probably due to the loss of the dendritic trees of Purkinje cells (Fig. 3) (36). In contrast, no Purkinje cell degeneration could be detected in younger Ngsk PrP-/- mice and old PrP+/+ and Ngsk PrP+/- mice (36). We also confirmed that the ataxia and Purkinje cell degeneration in Ngsk PrP-/- mice could be successfully rescued by introduction of the transgene encoding PrP^C (Fig. 3) (32). Similar cerebellar degeneration was subsequently reported in other lines of PrP-/- mice, such as Rcm0 PrP-/-

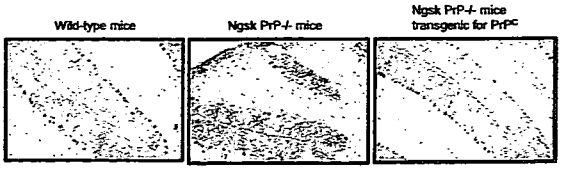

Fig. 3. Purkinje cell degeneration in Ngsk PrP-/- mice and Purkinje cells rescued in Ngsk PrP-/- mice transgenic for PrP^C. Purkinje cells are immunohistochemically stained using anti-calbindin antibodies and can be observed as brownish dots.

and Zrch II PrP-/- mice (5, 37). Taken together, these results indicated that the functional loss of PrP^C is responsible for Purkinje cell degeneration, although it remained unknown why the neurodegeneration was discrepant among different lines of PrP-/- mice.

In Zrch I PrP-/- mice, a part of the PrP open reading frame (ORF) was replaced with the neomycin phosphotransferase (neo) gene (Fig. 4) (26). In Npu PrP-/- mice, the neo gene was simply inserted into a unique site in the PrP-coding sequence (Fig. 4) (35). In contrast, in the ataxic lines of Ngsk PrP-/-, Rcm0 PrP-/-, and Zrch II PrP-/- mice, the entire ORF was completely deleted (Fig. 4) (5, 36, 37). It was therefore conceivable that in non-ataxic lines of Zrch I PrP-/- and Npu PrP-/- mice, some aspects of the normal function of PrP^C might remain intact because of incomplete disruption of the PrP allele. Consistently, it was reported that a fused mRNA consisting of the neo and the residual *Prnp* sequences was produced in their brains (26). Alternatively, it might be possible that the loss of PrP^C alone may not be enough to induce Purkinje cell degeneration, and that other factor(s), which are specifically associated with the ataxic lines of PrP-/- mice, together with the loss of PrP^C, are involved in the neurodegeneration.

Ectopic expression of PrPLP/Dpl associated with Purkinje cell degeneration

We found that, in the brains of Ngsk PrP-/- mice but not in Zrch I PrP-/- and PrP+/+ mice, the PrPLP/Dpl-coding exons were ectopically expressed as chimeric mRNAs with the residual non-coding *Prnp* exons 1 and 2 due to an abnormal intergenic splicing taking place between *Prnp* and *Prnd* (7). In Ngsk PrP-/- mice, due to lack of the 3' part of intron 2 including a splice acceptor, the pre-mRNA transcribed from the residual *Prnp* promoter could not efficiently undergo cleavage/polyadenylation at the end of *Prnp* (Fig. 5B). The unsuccessfully cleaved pre-mRNA was then elongated until the last

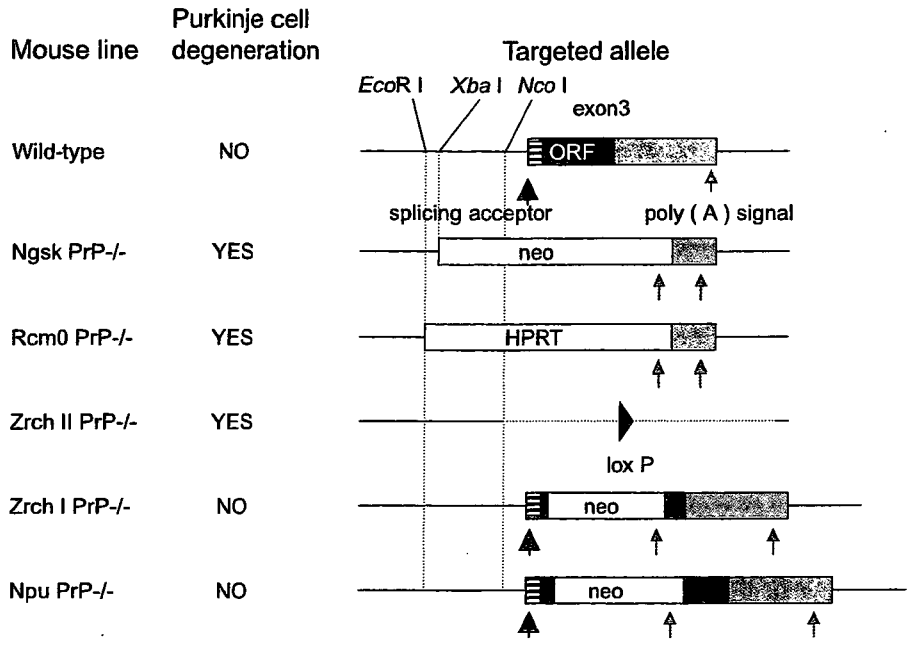

Fig. 4. Targeted PrP alleles among different lines of PrP-/- mice. In Ngsk PrP-/- mice, a 2.1-kb genomic DNA segment including 0.9-kb of intron 2, 10-bp of 5' untranslated region (UTR) of exon 3, the entire PrP ORF, and 0.45-kb of 3' UTR is replaced by the neo gene under the control of the mouse phosphoglycerate kinase (PGK) promoter. Rcm0 PrP-/- mice were generated by a similar targeting strategy utilized in Ngsk PrP-/- mice. The hypoxanthine phosphoribosyltransferase gene under control of the PGK promoter was used in Rcm0 PrP-/- mice as a selectable marker. In Zrch II PrP-/- mice, 0.27-kb of intron 2, the entire exon 3, and 0.6-kb of the 3' flanking DNA segment were targeted by a specific 34-bp *loxP* sequence. In these lines of PrP-/- mice, the entire PrP ORF is completely deleted. In contrast, Zrch I PrP-/- mice were generated by replacement of PrP codons 4-187 among a total of 254 codons with the neo gene under the control of the herpes simplex virus thymidine kinase promoter. Npu PrP-/- mice contain the disrupted *Prnp* alleles, in which the neo gene under the control of the mouse metalothioneine promoter was simply inserted into a unique *Knp* I site in the PrP-coding sequence.

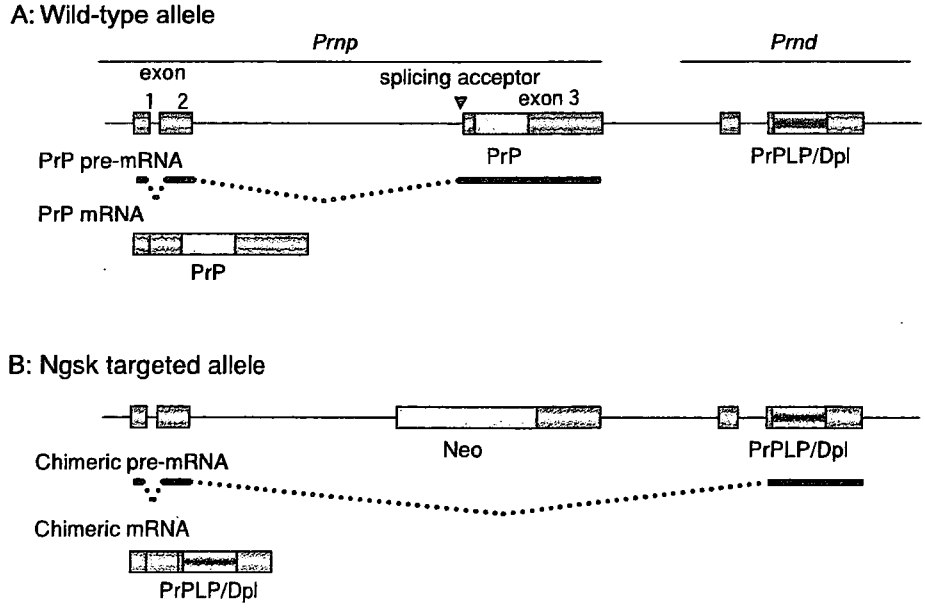

Fig. 5. Mechanism for the generation of PrPLP/Dpl-encoding chimeric mRNAs in Ngsk PrP-/- mice. In wild-type mice, the PrP pre-mRNA is normally cleaved and polyadenylated at the last exon of the *Prnp* (A). However, in Ngsk PrP-/- mice, due to lack of the 3' part of intron 2, the pre-mRNA transcribed from the *Prnp* promoter could not efficiently undergo cleavage/polyadenylation at the last exon of *Prnp*, being further elongated until the last exon of *Prnd*, and subjected to intergenic splicing between the residual *Prnp* exons 1/2 and the PrPLP/Dpl-coding exon (B). As a result, PrPLP/Dpl became abnormally expressed under the control of the *Prnp* promoter, leading to the ectopic expression of PrPLP/Dpl in the brains of ataxic lines of PrP-/- mice.

exon of *Prnd* and subjected to intergenic splicing between the residual *Prnp* exon 2 and the PrPLP/Dpl-coding exon, producing chimeric mRNAs consisting of the *Prnp* exons 1 and 2 and the PrPLP/Dpl-coding exons (Fig. 5B). As a result, *Prnd* became abnormally regulated under the control of the *Prnp* promoter in Ngsk PrP-/- mice and PrPLP/Dpl was ectopically expressed in the brains, especially in neurons and glial cells where the promoter is very active (7). Similar ectopic expression of PrPLP/Dpl was subsequently reported in other ataxic lines of PrP-/- mice, Rcm0 PrP-/- and Zrch II PrP-/- mice (5, 37). Taken together, these results indicate that the ectopic expression of PrPLP/Dpl in the absence of PrP^C might be responsible for Purkinje cell degeneration in the ataxic lines of PrP-/- mice.

PrPLP/Dpl in the absence of PrP causes Purkinje cell degeneration

To investigate the possibility that ectopic expression of PrPLP/Dpl in the absence of PrP^C could cause the Purkinje cell degeneration, we generated transgenic mice, referred to as tg(N-PrPLP/Dpl) mice, in which PrPLP/Dpl was specifically expressed in nearly all neurons including Purkinje cells under the control of the neuron-specific enolase promoter, and subsequently crossed them with the non-ataxic line of Zrch I PrP-/- mice (38). Tg(N-PrPLP/Dpl) 32 mice expressed PrPLP/Dpl in the cerebellum at a level about 1-2 times more than that of Ngsk PrP-/- mice, developing ataxia at 58±15 days on the Zrch I PrP-/- background (Table 3). Purkinje cells were markedly decreased in number due to the degeneration in these ataxic tg mice. In contrast, neither ataxia nor Purkinje cell degeneration could be detected in the tg mice carrying the PrP+/+ background (Table 3). Another tg(N-PrPLP/Dpl)25 mouse line, in which PrPLP/Dpl was expressed in the cerebellum at a level less than a quarter that of Ngsk PrP-/- mice, also developed ataxia and Purkinje cell degeneration at 359±52 days on the Zrch I PrP-/- background but not on the PrP+/+ background (Table 3). These results clearly showed that the ectopic expression of PrPLP/Dpl is neurotoxic in the absence of PrP^C, causing the Purkinje cell degeneration, and that the neurotoxicity of PrPLP/Dpl is antagonized by PrP^C.

We also produced another type of tg mice, tg(P-PrPLP/Dpl) mice (38). In these tg mice, the expression of PrPLP/Dpl was specifically targeted to Purkinje cells under the control of the Purkinje cell protein-2 promoter (38). Like tg(N-PrPLP/Dpl) mice, tg(P-PrPLP/Dpl) mice developed ataxia and Purkinje cell degeneration on the Zrch I PrP-/- background but not on the PrP+/+ background (38). Tg(P-PrPLP/Dpl)26 and 27 mice showed ataxia at 268±28 and 167±13 days after birth, respectively, on the Zrch I PrP-/- background (Table 3). These results clearly indicate that PrPLP/Dpl ectopically expressed on Purkinje cells is itself neurotoxic to the cells.

Stoichiometrical antagonism between PrP and PrPLP/Dpl in neurodegeneration

The times to the onset of ataxia in tg(N-PrPLP/Dpl) mice were inversely correlated with the expression levels of PrPLP/Dpl (38). Tg(N-PrPLP/Dpl)32 mice expressed more PrPLP/Dpl in the cerebellum and developed the ataxia earlier than tg(N-PrPLP/Dpl)25 mice (Table 3). In contrast, the levels of PrP^C were correlated with the times of the onset. Tg(N-PrPLP/Dpl)32 and 25 mice showed significantly retarded onset of the ataxia on the Zrch I PrP+/- background, compared with the Zrch I PrP -/- background (Table 3). Thus, these results indicate that PrPLP/Dpl and PrP^C stoichiometrically antagonize each other to induce Purkinje cell degeneration.

N-terminal domain of PrP antagonistic for PrPLP/Dpl

PrP^C possesses the OR region-containing N-terminal domain whereas PrPLP/Dpl lacks the corresponding domain. It is therefore conceivable that the N-terminal domain might be important for PrP^C to antagonize against the PrPLP/Dpl neurotoxcity. To investigate the possibility, we introduced PrP with a deletion of the N-terminal residues 23-88

Table 3 PrPLP/Dpl stoichiometrically antagonizes PrP^C to induce ataxia

Tg	lines	Onset of ataxia on different genetic backgrounds (mean±SD days)		
		Zrch I PrP-/-	Zrch I PrP+/-	Wild-type
Tg(N-PrPLP/Dpl)	25	359±52	495±86	<600
	32	58±15	259±48	<600
Tg(P-PrPLP/Dpl)	26	268±28	463±81	<600
	27	167±13	391±108	<600

into Ngsk PrP-/- mice (39). As expected, the deletion mutant PrP failed to rescue Ngsk PrP-/- mice from ataxia and Purkinje cell degeneration (39). Ngsk PrP-/- mice expressing the deletion mutant developed ataxia and Purkinje cell degeneration on a time course identical to that of non-transgenic Ngsk PrP-/- mice (39). These clearly indicate that the N-terminal residues 23-88 are important for PrP^C to antagonize the neurotoxicity of PrPLP/Dpl. The N-terminal residues 23-88 include most of the OR region. Thus, it is suggested that this OR region could be important for PrP^C to protect Purkinje cells from PrPLP/Dpl-induced degeneration, although it remains to be investigated which region in the deleted N-terminal domain could be essential for the neuroprotection of PrP^C against PrPLP/Dpl.

We also introduced PrP carrying a familial prion disease-associated mutation (E199K) into Ngsk PrP-/- mice (39). Interestingly, these mice developed no ataxia and Purkinje cell degeneration (39), showing that the mutant PrP was fully functional for antagonizing the PrPLP/Dpl-induced neurotoxicity, suggesting that other disease-associated mutant PrPs are also functionally competent.

PRP AND PRPLP/DPL IN ISCHEMIC NEURONAL CELL DEATH

To assess whether PrP^C and PrPLP/Dpl could be involved in other types of neuronal cell death, we subjected Zrch I PrP-/- and Ngsk PrP-/- mice to transient forebrain ischemia (40). Interestingly, male Zrch I PrP-/- mice were very susceptible to the ischemia compared to control PrP+/+ mice, developing marked apoptosis in the hippocampal CA1 region (40). McLennan, *et al.* also reported that permanent occlusion of the middle cerebral artery increased the infarction volume in male Npu PrP-/- mice without no ectopic expression of PrPLP/Dpl in neurons (41). However, no apoptotic cell death could be detected in the CA 1 of female Zrch I PrP-/- mice (40). Taken together, these results indicate that PrP^C is involved in neuroprotection against brain ischemia, and that the neuroprotective function of PrP^C is masked by female-specific neuroprotective factor(s).

We also showed that, in contrast to Zrch I PrP-/- mice, both male and female Ngsk PrP-/- mice exhibited severe ischemic damage to CA1 neurons (40). Since Ngsk PrP-/- mice ectopically express PrPLP/Dpl in neurons, it is therefore conceivable that PrPLP/Dpl might counteract the female-specific neuroprotective function, thereby increasing the susceptibility of PrP^C-deficient neurons to ischemic insults.

ROLES OF PRP AND PRPLP/DPL IN NEURODEGENERATION

Neurotoxic PrPs

PrPLP/Dpl is a homologue of the C-terminal part of PrP^C. Interestingly, it was shown that the N-terminally truncated PrPs, PrPΔ32-121 and PrPΔ32-134, induced ataxia and cerebellar degeneration characterized by marked granule cell death in Zrch I PrP-/- mice and the neurotoxicity of these truncated PrPs was antagonized by the expression of full-length PrP^C (42). No Purkinje cell degeneration was observed in these mice because the truncated PrPs were not expressed in Purkinje cells of these mice due to the limited activity of the promoter used (42). Consistently, it was demonstrated that ataxia and Purkinje cell loss could be induced in Zrch I PrP-/- mice when PrPΔ32-134 was targeted to Purkinje cells (43). PrPΔ32-121 and PrPΔ32-134 encompass the homologous C-terminal part of PrP^C to PrPLP/Dpl. It is therefore very likely that PrPLP/Dpl and the truncated PrPs might use the same or a very similar molecular mechanism to execute the neurotoxicity.

Cis- and trans-neuroprotective function of PrP against PrPLP/Dpl

In contrast to neurotoxic PrPΔ32-121 and PrPΔ32-134, it was shown that PrPΔ23-88 was not neurotoxic, causing no Purkinje cell degeneration in Zrch I PrP-/- mice (39), suggesting that the neurotoxicity of the C-terminal domain of PrP^C is blocked by a cis-element(s) present in the region between the residues 89 and 121. This region, overlapping with the central hydrophobic part, is reported to comprise part of the binding sites for the heat shock protein, stress-inducible protein 1, and the extracellular matrix constituent glycosaminoglycans (44, 45). It is therefore possible that interaction of PrP^C with these molecules might be involved in the cis-inhibition of the neurotoxicity of the C-terminal domain of PrP^C. In contrast, PrPLP/Dpl and the truncated PrPs are unable to interact with these molecules, therefore acting as neurotoxic proteins.

PrP^C also neutralizes the neurotoxicity of PrPLP/Dpl and the truncated PrPs in trans, rescuing from