24, 27, 29]. The present study demonstrated that the presence or absence of TX was one of the determinants for tau2 in reacting with its target epitope. Because tau protein present in normal brain tissue is hardly visualized on immunohistochemistry on fixed sections [16, 26], pathological modifications of tau, such as phosphorylation in Alzheimer's disease (AD) and DS brains, have been considered to be prerequisite for immunohistochemical visualization of tau on fixed histological sections [21]. Biochemical analysis of the present study, however, demonstrated that tau2-immunoreactive bands were detectable mainly in the TS-fraction of MSA brains (Fig. 6A, B); it is reasonably assumed that tau2 IR on GCIs seen on histological sections corresponds to these tau2-immunoreactive bands in the TS fraction. These findings signify that modification of tau protein in MSA brains is different from that of AD, usually associated with a mobility shift and more resistant to solubulization, which represent pathological changes probably linked to abnormal phosphorylation. In other words, modification of tau in MSA brains is not related to its phosphorylation, but is sufficiently visualized on fixed brain sections, even though normal tau similarly detected in TS fraction [9] is indistinguishable on immunoblot from modified tau in MSA. This is in accordance with a previous report, which clarified that tau epitopes on GCIs were usually limited to those detected with phosphorylation-independent antibodies and similar to normal adult tau [8]. Because histological sections from normal bovine brain, even containing tau with an extremely high affinity to tau2 on immunoblot [37], failed to exhibit apparent immunohistochemical labeling with tau2, immunohistochemical visualization of tau2 epitope requires some pathological modifications of tau proteins rather than its abundance.

Tau2 was initially raised against bovine tau [22], and its epitope was mapped to Ala95–Lys119 of bovine tau [37]. It was reported that Ser101 is crucial for its affinity to tau2, and its replacement with Pro, as in human tau, is associated with a significant decrease in its affinity to tau2 [37]. Higher affinity of modified tau protein in NFTs to tau2 is explained if this Pro undergoes some conformational change mimicking this Ser-like conformation seen the bovine brain. Because the antigen peptide mimicking this Ser-like conformation absorbed tau2 IR on GCIs (Fig. 1B), modified tau proteins in GCIs and NFTs share this conformational state at the tau2 epitope. It indicates, however, that this conformational change of tau2 epitope in GCIs of MSA is not necessarily linked to phosphorylation of tau.

Co-presence of TX with tau2 abolished these tau2-immunoreactive bands in a reversible fashion on brain homogenates, irrespective of the diagnoses (Fig. 7B, C), while affinity of tau2 to bovine tau was less affected by TX (Fig. 7B, lane 3), probably because the original conformation of Ser101 is retained. This relative resistance to TX was shared with NFTs only when observed on fixed histological sections of DS brain, suggesting that organization of tau protein into PHF confers some stability of this tau2 epitope. Because this relative resistance to TX was not observed with either solubilized PHF tau or extracted tau from MSA brain on SDS-PAGE, it is probably related to the fibrillary structure remaining as NFTs on fixed histological sections, which should, however, have been destroyed during solubilization for SDS-PAGE.

Although this modification of tau2 epitope is shared between NFTs of DS and GCIs of MSA, immunohistochemical visualization with other anti-tau antibodies, which uniformly immunolabels NFTs, was unsuccessful on GCIs [8]. Because the relative intensities of tau-immunoreactive bands, detected either with tau2 or pool2, did not differ greatly between NFTs and GCIs, modification of tau and its extent, rather than its abundance, probably will explain this difference in immunohistochemical features between these conditions. These immunohistochemical and biochemical features of GCIs, as well as their susceptibility to TX, are indistinguishable from that observed on microglial cells around ischemic foci, whereas these microglia never develop argyrophilia or fibrillary structures [34].

We have demonstrated with immunoelectron microscopy that tau2 epitope is localized to fibrillary structures typical of MSA. Although the tau2 epitope is represented on these fibers, it remains to be settled whether tau protein is one of the major constituents of these fibrillary structures in GCIs [1, 15, 24, 27]. Most of tau proteins in MSA brain are extractable with TS, suggesting that they are not tightly integrated into these fibrillary structures. This does not, however, exclude the possibility that the tau protein may play some roles in the formation of fibrillary structures composed, for example, of α-synuclein, because colocalization of tau and α-synuclein is rather common in various types of inclusions [4, 6, 25, 30]. Another feature that characterizes GCIs is their argyrophilia, also shared with NFTs. Although the molecular basis for the argyrophilia remains to be clarified, argyrophilia in GCIs and in NFTs is considered to be linked to their common fibrillary composition [24], because electron microscopic studies demonstrated that these fibrils are both decorated by the silver granules [13, 28]. Therefore, GCIs share immunohistochemical features with microglia around ischemic focus [20, 34, 35] and argyrophilic fibrillary features with NFTs [21]. This modification of tau2 epitope seen in GCIs may represent an early event in their formation, as observed with early stage of NFT formation not associated with fibril formation or with immunohistochemical visualization of other tau epitopes [7]. Phosphorylated tau epitopes on GCIs has been reported in a case of MSA with an exceptionally long duration of the disease (19 years) [25]. The shorter disease duration in our series may explain the observed difference, suggesting that phosphorylation of tau, possibly in GCIs, is one of the late consequences rather than a primary event triggering GCI formation. More importantly, phosphorylation of tau does not lead to formation of PHF in MSA brains [25], suggesting again that tau proteins are not one of the principal constituents of fibrillary structures in GCIs.

Selective immunohistochemical visualization of tau2 epitope not associated with other tau epitopes has been

described in some cases with degenerative process characterized by ubiquitin-positive neuronal inclusions [10, 14]. We do not yet know whether this selective immunohistochemical visualization of the tau2 epitope shared by these conditions and GCIs is similar to that observed on microglial cells around ischemic foci [20, 34, 35] or whether this has some additional relevance to each degenerative process [10, 14]. Because tau-positive structures associated with brain ischemia (Alz-50-positive neurons [33] or tau2-positive microglia [20, 34, 35]) never develop NFTs, it is expected that further steps or distinct cascades of tau modification is associated with fibrillary structures of GCIs, a process probably independent of PHF formation.

Although simple immunohistochemical visualization of tau is not sufficient to distinguish different types of tau deposits, selective modification of tau2 epitope and the sensitivity of tau2 epitope to TX, as demonstrated in this study on GCIs, will provide an additional feature distinct from NFTs. This simple method will potentially provide information on conformational state of deposited tau proteins and help in distinguishing different pathological conditions or staging disease progression. Further studies will clarify the molecular basis to explain possible conformational changes of tau2 epitope during formation of GCIs and its relation to α-synuclein. Distinction from other degenerative tauopathies will be necessary to disclose modifications of tau protein specific for each pathological process.

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CAGリピート病（ポリグルタミン病）の最近の話題

共通の病態と治療へのてがかり

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Key words
polyglutamine, neuronal intranuclear inclusion, CAG repeat, therapy, review

はじめに

遺伝性脊髄小脳変性症（spinocerebellar degeneration: SCD）やHuntington病（HD）は失調や不随意運動等の神経症状のほかに，知能低下，人格変化，自殺企図などの精神症状も呈し得る器質的疾患である。これらの疾患では

1. 単因子の優性遺伝性疾患として矛盾がなく，世代を経る毎に発症年齢は低く，症状もより激烈になる。(anticipation)
2. 発症に関連する遺伝子のcoding regionにCAGの3塩基モチーフの繰り返しがあり，このCAG repeat数の延長が上記1と関連する。
3. 延長したCAG領域を含む遺伝子産物が，主に神経細胞の核内に封入体（Neuronal Intranuclear Inclusion: NI，図1）を形成する。

という共通した特徴を持つ一群がある。これらの疾患では遺伝子の発現，NI形成から変性に至る病態の少なくとも一部は共通していると考えられ[13)]，CAG repeat病として多方面からの研究が精力的に行われている。以前は様々な名称で報告されてきた疾患を，遺伝子型からまとめたのが表1である。本稿では主に剖検脳にみられるNIからみた細胞変性機序を中心に，最近の研究の進歩をまとめ，治療の試みを紹介する。

1. 臨床像と病理像の関連

変性過程の原因が長らく不明のまま，神経細胞の脱落とグリア細胞の増生所見，およびその分布が記載の対象となってきたのはCAG repeat病も例外ではない[16, 37)]。HDの病変の主座は尾状核・被殻にあり，その病理変化の程度を基準にしてVonsattelらは病期を分類している[40)]。HDの剖検脳で尾状核や被殻の神経細胞密度を計測し，年齢で補正すると，CAG repeatの数との間には逆相関があり，神経細胞脱落は延長したCAG repeatの長さが長い程強いという[7)]。従来から随意，不随意の運動障害はこれら基底核病変に対応すると考えられているが，HDにみられる人格の変化を含む精神症状や知能障害も基底核病変で十分説明できるかは異論がある[16, 30, 37)]。実際HDでは大脳皮質にも進行性の萎縮があり，正常成人で1300g以上の脳重が長期経過したHD例では1000g以下に減少することも知られており，大脳皮質に病変の主座をもつピック病やアルツハイマー病（AD）脳に匹敵する程強い萎縮を呈

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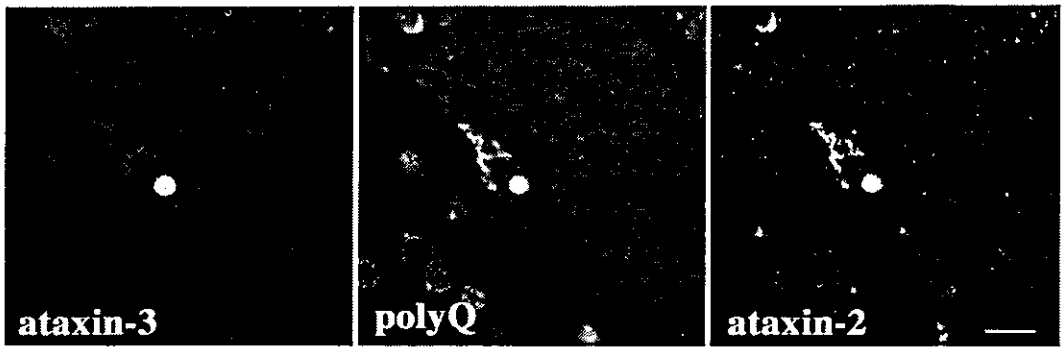

図1　SCA1橋核神経細胞にみられた核内封入体の蛍光3重染色
封入体に集積する延長したpolyQはataxin1に由来するが，ここには正常のataxin2，ataxin3が共局在している。
Acta Neuropathol 2001:109:149-52より改変

表1　CAG repeatの延長が疾患の発症に関連する既知の疾患

疾患名	遺伝子座	遺伝子	翻訳産物	CAGリピート数 正常範囲	CAGリピート数 病的伸長
SCA1	6p23	SCA1	ataxin1	6-36	39-83
SCA2	12q24.1	SCA2	ataxin2	15-31	34-400
SCA3/MJD	14q21	SCA3/MJD	ataxin3/MJD1	12-40	55-86
SCA6	19p13.1-p13.2	CACNAIA	α 1A Ca channel	4-16	20-33
SCA7	3p21.1-p12	SCA7	ataxin7	4-19	37-300
SCA12	5q31	SCA12	PP2A	6-26	66-78
SCA17	6q27	SCA17	TBP	30-42	45-63
DRPLA	12p	CTG-B37	atrophin1	3-36	49-88
HD	4p16.3	HD	huntingtin	10-26	36-121
SBMA	Xq11-q12	AR	androgen receptor	-34	38-

Gene Review (http://www.genecllinics.org)より改変
SCA: spinocerebellar ataxia, MJD: Machado-Joseph disease, PP2A: protein phosphatase2A, TBP:TATA binding protein, DRPLA:dentatorubral pallidoluysian atrophy, HD: Huntington disease, SBMA: spinal and bulbar muscular atrophy

し得る。正常線条体全体の重量が高々200g程度とすると，脳重の減少は大脳皮質等のより広範囲な変化も加わっていると考えざるを得ない。ところがこのような例の大脳皮質を顕微鏡的に観察しても，グリア細胞の増生が驚く程軽い点でAD等とは異なり，HD脳の大脳皮質の病理形態学的特徴と考えられている[16,30,37]。

Dentatorubral pallidoluysian atrophy（DRPLA）ではその名の通り，変性の主座は歯状核・赤核系とルイ体・淡蒼球系にわたるが，HD同様脳重が著しく小さい例があり，限局した部位の萎縮のみでは脳重の減少を到底説明できない[13,24]。上記の変性部位を別にすると，脳全体は均等に萎縮している。この特徴を我々は“小造り”と形容しているが[13]，同様の傾向はSCA2[18]，SCA17[25]等でも認められる。細胞の萎縮が明らかなのにグリアの増生は目立たない点は，一旦完成された構造が破壊されるという従来の変性の考え方では説明しがたい所見で，程度の差はあれCAG repeat病に特有の特徴と考えられる。

2. 核内封入体とその意義

HDやSCA3の神経細胞にNIが同定されると，

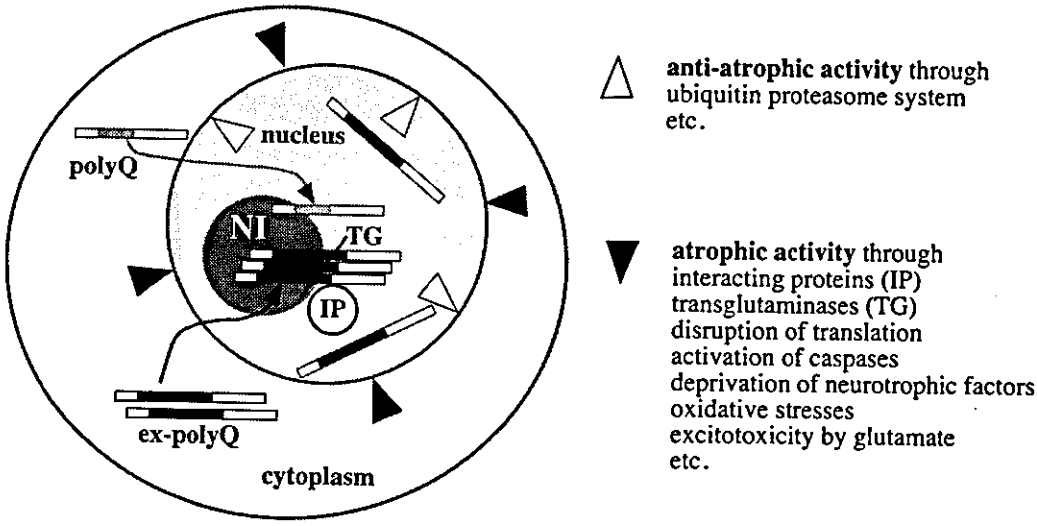

図2　CAGリピート病の核内封入体と細胞内異常

細胞質で翻訳された延長したポリグルタミン蛋白（ex-polyQ）と正常ポリグルタミン蛋白（polyQ）が核内に入るが，トランスグルタミナーゼ（TG）はこれらの蛋白を架橋し封入体（NI）形成を促進しているという。Ex-polyQに親和性を有する因子（IP: interacting proteins）を介し，萎縮を促進（atrophic activity）あるいは萎縮に拮抗（anti-atrophic activity）する機構が活性化される。

他のCAG repeat病にも相次いで同様の病的構造物が見出された。Repeatの延長の程度が僅かでも臨床症状を呈するSCA6を除いて，これまで剖検脳が観察されたCAG repeat病のほとんどでNIが確認されている。CAG repeat病のNIはSCA1，SCA17でeosin好性，HDでCongo red陽性等の報告があり疾患により一定しないが，通常の染色で同定できるものはヒトCAG repeat病の剖検脳では一部に留まる。これらのNIはユビキチン陽性であり，延長したCAG repeatの翻訳産物である延長したポリグルタミン鎖（polyQ）を含む遺伝子産物が一部のNIに含まれている（図1）[39]。HD発症前のcarrierの剖検例にもNIは認められ，病的過程は早い時期から進行している[8]。CAG repeat病の剖検脳には多かれ少なかれ，神経細胞の脱落よりなる変性過程とNIが見られ，しかもNIには関連遺伝子の産物が集積するので，NIのある細胞は変性の影響をより強く受けるとの仮説が当初より当然のことと考えられた。確かに，延長したCAG repeatを含む遺伝子を培養細胞やマウス神経細胞に発現させるとユビキチン陽性のNI様構造物とアポトーシスによる細胞死が誘導されることが多くの実験系や動物モデルで確認されており，細胞集団全体で見た場合はこの仮説は実験的な裏付けを得るように思われる。

一方NIに集積するユビキチンは細胞内の異常な蛋白を処理する過程に関与する分子であり，他にもHSP70等のproteasomal effectorがNIに局在していることから，NIは異常蛋白である延長したpolyQ（expanded-polyQ=ex-polyQ）を含む遺伝子産物を処理する過程で形成されるとの考え方がある。しかしNIの出現頻度は疾患や部位によって異なる。CAG repeat病の多くは小脳失調を呈し，その程度には疾患による違いはあるが，小脳病変を持つ疾患が多い。我々はSCA1，SCA2, SCA3, DRPLAの小脳皮質を系統的に検索したが，病変の程度に関わらずNIを持つPurkinje（Pj）細胞は全くみられなかった[19]。変性の初期に限ってNIがPj細胞に出現していた可能性は否定できないが，逆にCAG repeat病のPj細胞はNIを形成できない分だけ変性に陥り易いという推論も可能になる。事実SCA3剖検脳の橋神経細胞の断面積を測定すると正常に比して

小さいが，NIを有する細胞はNIを有さない群に比して有意に大きい[38]。NI形成が細胞変性を促進するという仮説では説明できない所見であり，少なくともCAG repeat病のヒト剖検脳にみられるNIは細胞変性を一方的に促進するのではなく，拮抗する機能とも結びついていると考えるほうが説明しやすい。同様の傾向はDRPLAの小脳顆粒細胞[34]やSCA1脳の橋核神経細胞でも共通しており，HD脳でもNIの密度と神経変性の程度は相関しないことが知られている[21]。CAG repeatの延長をもつataxin1を導入したtransgenic miceではNI様の封入体が形成されるが，ユビキチンによる異常蛋白処理に必須の酵素であるE6-AP-ubiquitin ligase活性をこのマウスで低下させると形成されるNIの数が減少すると同時に変性細胞は増加するとの報告がある[4]。CAG repeat数の延長したhuntingtin（m-htt）を導入した細胞でも同様の現象がみられ，誘導されたアポトーシスをcaspase阻害剤で阻止するとNIの形成は逆に促進されるという[31]。以上の様に，CAG repeat病にみられるNIが直接細胞障害につながるという当初の仮説だけでは説明できない所見も次第に集積されている。

3. 封入体に局在する分子と核内での働き

延長したCAG repeatを持つ遺伝子の産物がNIの構成要素であることは，多くのCAG repeat病で明らかにされているが，その他にも様々な分子がNIにはとりこまれており，NIの機能や形成過程に関与している可能性がある。

延長したCAG repeatを持つ遺伝子産物自体はその全長または一部がNIに局在するが，同時に延長していない他の正常polyQ蛋白もNIに局在する[39]。例えば，SCA3脳のNIにはCAGの延長したataxin3が含まれるが，CAG延長のないpolyQ蛋白（ataxin2やTBP）も同時に含まれており，polyQ蛋白は非特異的にNIにとりこまれていると考えられる。したがってSCA3脳のNIは正常ataxin2に加えておそらく正常のataxin3もふくむと予想され，NIに局在するpolyQ蛋白の種類から，関連する遺伝子異常の違いを区別することはできない点注意を要する（図1）。これらのポリグルタミン蛋白の機能は不明だがataxin3は老化や肝性脳症脳の黒質神経細胞核内に多数出現するMarinesco小体（MB）[20]やCAG repeatの延長との関係が明確でないNeuronal Intranuclear Hyaline Inclusion Disease[35]のNIにも高率に存在し，各種の封入体に共通する機能を担っている分子である可能性がある。

Monoclonal抗体1C2はex-polyQに特異的に親和性を有し，NIを陽性に染色するが，標本を蟻酸で処理するとCAG repeat病脳では核質全体がdiffuseに染色される神経細胞が観察される。蟻酸処理による1C2染色性の変化は，核質内とNIの中とではex-polyQの存在様式（conformationや濃度）の差を反映している可能性がある。1C2のdiffuse nuclear stainingは通常の染色で変性が見られない部位や，NIを持たない細胞にも見られることから，NI形成の前に起こる変化であるとの考え方がある（図2）[42]。また正常神経細胞の核内にはPML（promylelocytic leukemia protein）bodyと呼ばれる微細な構造があり，蛋白の過剰産生や異常蛋白の産生にともなって，より大きな構造に変化するため，これらの蛋白の処理過程に関与すると思われる。実際一部のNIやMBはPML陽性であり，正常の細胞でみられた微細な構造はこれらの封入体の形成につれて消失する。従って，核内の蛋白の量的・質的異常の少なくとも一部はPMLbodyによって処理されていると推察される[20]。一方1C2陽性の構造は細胞質にも存在し，電子顕微鏡的にはlysosomeに局在するという[43]。CAGrepeat病でex-polyQが病的過程を引き起こしているとすると，その細胞内での動態を把握することが，治療法の開発を念頭においた病態の把握に不可欠であり，今後の検討が待たれる。

4. 核内の機能異常と変性過程

CAG repeat病の発症に関連する異常遺伝子の発現は中枢神経内でubiquitousなものが多く，遺伝子の発現パターンは対応する疾患の病変分布とは一致しない。一方で臨床症状や病変分布は一定のパターンをとり，系統変性症としての疾患特異的な病変分布がある遺伝子異常からどのように引き起こされるのかは全く判っていないと言って良い。ex-PolyQを含む遺伝子産物が病的な作用を有し，かつex-polyQの発現自体は部位特異的でないとすると，それ以外の要素がその病的作用の発現に介在していることが推測される。

核内ではDNAからRNAへの転写が盛んに行われており，転写調節に関わる因子の幾つかはNIに沈着している。とするとこの転写調節因子によって維持されている正常機能に障害がおこるのではないかという仮説がある。実際，転写調節因子の一つであるTATA binding proteinはCAG repeatを有し，その病的延長がSCA17の発症に関連していることが明らかとなった[25]。TAFII130はDRPLA蛋白と結合する転写因子の一つで細胞にex-polyQを導入して形成されたNIに集積した場合，CREB依存性転写活性が低下し細胞死が誘導されるが，これらの因子を強制発現すると細胞死が阻止されるという[33]。ex-polyQに選択的に結合する蛋白として同定されたPQBP1は延長したataxin-1との結合を介して，RNA - polymeraseIIの機能を阻害し得ることも示された[26]。

このようにNIは転写調節障害の起点となる一方で，ubiquitin-proteasome系の作用の場でもあり得る。前者が細胞障害，後者が細胞保護に働き，両者ともpolyQにより惹起されているとすると，CAG repeat病の細胞ではすくなくとも2つの相反する過程が起こっていることになり，一元的な解釈は難しいと思われる（図2）。同一の細胞の中で拮抗するこれらの過程の総和として変性が長期に進行するのがCAG repeat病では共通する特徴と考えられ，最初にのべたグリア細胞の反応の乏しさや小作りといった形態的特徴もこのような視点から考えると理解しやすいのではないかと我々は考えている。

5. 内因性精神疾患とCAG repeat病

HDでは病初期に不随意運動が目立たず精神症状が前景に立つ場合がある[16, 30, 37]。DRPLA, SCA6, SCA17等でも当初は精神症状が全景に立つ例の報告があり，発症初期にはいずれも内因性の精神疾患と臨床的に区別が難しい場合があることに注意すべきである。またDRPLAでは白質のdiffuseな信号強度の変化がMRIで見られる例があり，鑑別診断の助けとなる[1]。これらの家系では世代を経る毎に，発症年齢が若年化する（anticipation）のが一般的で，親の世代が発症年齢に達しないで死亡した場合，家族歴が明瞭でない。従って他の神経症状が無くてもこれらの疾患を念頭において診断治療を進めることで，診断が明らかになる場合がある。

逆に精神症状のみで終始する各種の内因性精神疾患（統合失調症，周期性疾患等のmajor psychoses）でも，家族性で，世代を経る毎に発症年齢が若年化する傾向を持つ家系が以前から知られている。この特徴はこれまで報告されたCAG repeat病と共通しており，内因性精神疾患に関連する遺伝子異常があるとすればその一部はCAG repeatの伸長ではないかという推測を基にした研究が積み重ねられている。しかし，これまでのところこれらの疾患に関連することが確定したCAG repeatの伸長はなく，これらの剖検脳でCAG repeat病にみられるようなNIを同定したとの報告も無い。

6. CAG repeat病の治療

CAG repeat病が共通の発症機序を有するとすれば，共通の治療戦略が可能になることが期待される。とくに遺伝子検査による発症前診断が

可能となった現在，この異常から引き起こされる過程を少しでも阻止する方法を見出すことが，急務となる。また，遺伝子導入などによるモデル動物が作製され，薬剤投与の影響を個体レベルでも観察できるようになった点で急速な進歩が期待される[22]。形成された病変は不可逆であるかのように考えられてきたが，polyQに病的延長のあるhuntingtinの発現を途中で低下させると，mouse脳に一旦形成されたNIでも消失したという[44]。脳に形成された病変といえども可逆的であることが明らかにされたわけで，治療法開発への期待は一層高まったと言える。本稿では，症状の安定化等を対症的に狙う従来の薬物療法とは別に，なんらかの形で病態へ介入して変性過程自体に変化を加えようとする試みの幾つかを紹介する。

1）異常遺伝子の転写産物への介入

異常伸長したCAG repeatが転写された後，蛋白に翻訳されて病的機能を発揮するとすれば，転写産物であるRNAを特異的に阻害することで，その後の病的過程が抑制されることが期待される。当初antisense oligonucleotideやRNA干渉法を用いて転写を抑制することが考えられた。最近ではribozyme, DNA enzyme等，より小さな分子で，十分な特異性を保ちつつ対象の病的塩基配列を効率的に置き換える[28]あるいは切断する[45]核酸sequenceがみいだされ，実用化されれば症状の進行や発症を抑制し根治に結びつく可能性がある。しかしこれらの核酸鎖を安全に生体の細胞の核内へ十分量持ち込むこと，この核酸鎖が異常伸長のない正常alleleや他のRNAに影響を与えないこと，切断されたmRNA鎖が病的作用を持たない事等が治療の前提となり，技術的にも解決すべき問題は残されている。

2）Transplantation

おもにfetal tissueから得られた線条体細胞を変性部位に移植して，脱落した細胞の機能を補おうとする試みである。移植後の拒絶反応を抑制できた例ではtransplantとrecipientの組織間にsynapse形成がみられ，機能的にも有効な細胞が形成されるとの観察がある。臨床的効果については報告により一定しないが[9]臨床症状の改善は移植部の組織片の代謝活性がPETで亢進している例で明らかという報告もある[2]。今後神経幹細胞等の技術的改良により治療がより容易になる可能性も期待される。しかしこれらの疾患は多系統に変性が起こるのが通例で，HDにしても基底核病変に加え大脳皮質の萎縮も相当に強く，また前景にたつ症状も患者さんによって異なる[16, 37]。変性部位がほぼ限局し，症状のばらつきも比較的少ないパーキンソン病で一定の成功をおさめている本治療法が，変性の範囲がより広くまた症状も多彩なHDに対してどのように適応するのが最も効率的か今後検討を要する。さらに病変の広がりが大きなDRPLAやその他のSCDsでは局所的なtransplantationのみでは対応し難いという限界がある。

3）栄養因子

神経変性過程を栄養因子の相対的不足からとらえ，それを補うことで治療的介入を行うという発想はCAG repeat病に限らないが，治療的成功にいたったと言える例は乏しい。HD脳ではbrain-derived neurotrophic factor（BDNF）が低下していることが指摘されており，線条体由来の培養細胞にex-polyQを含むhuntingtin（htt）をtransfectさせるとBDNFの低下にともない，細胞死が誘導されやすくなるという[46]。面白いことに正常のhttを強制発現すると，BDNFの上昇に伴い細胞死は逆に抑制される。NIには正常のhttも集積しているとすると（図1），正常httの機能部位での枯渇が起こっている可能性があり，これが細胞死を一層促進する可能性がある。

4）ポリグルタミン蛋白の凝集抑制

Ex-polyQを含む蛋白が凝集してNIを形成するが，その過程でpolyQを基質とするtransglutaminase（TG）がペプチド間を架橋し，線維構造を強固にする可能性が指摘されている[12]。cystamineはin vitroでTG活性を選択的に阻害してpolyQ発現にともなって形成された凝集体形成を抑制する。HD miceに腹腔または経口からcysta-

mineを投与すると中枢神経系に取り込まれ，脳で亢進したcystamine活性を抑制し，運動障害等も抑制されるという[5,14]。ただし形成されたNIはcystamine投与により減少するという報告[5]としないという報告[14]があり，TGがNI形成を抑制する以外の経路でも変性過程に影響を与えている可能性がある。

Ex-polyQに結合するペプチドを11アミノ酸からなるphage display libraryからscreenすると，その内の一つ（QBP1, SNWKWWPGIFD）は合成polyQの会合をin vitroで抑制するだけでなく，培養細胞内でもNI形成と細胞死の両者を抑制するという[23]。その他にもex-htt（Q51）に親和性があり凝集を抑制する物質（Congo red, thoflavine S, chrysamine G）や抗体（1C2）が同定され，Congo redは培養細胞レベルでもNI形成を抑制するという[10]。分子量の小さな分子を生体内の病変形成部位で反応させることができれば，NI形成過程への治療的介入が可能になるかもしれない。

5）細胞障害をrescueする

神経変性の分子機構については，アポトーシス，ミトコンドリア機能異常，酸化ストレス，excitotoxicity等様々な仮説が原因遺伝子の同定以前から唱えられてきたが[36]，変性疾患の系統性を十分に説明し，かつ十分な治療効果が確定したものは少ない。CAG repeatの病的延長がこれらの系に直接あるいは間接に影響を与えている可能性があるが，細胞変性，細胞死に共通した過程でこれらの異常が起こるのかもしれない。いずれにしても神経細胞の障害をrescueする効果が期待できる薬剤の候補があげられており，モデル動物等での検討で一定の効果がみられているものがある。

a. Caspasesはアポトーシスを介した細胞死で活性化されるが，HD脳やHD-transgenic mice脳でもその活性は上昇している。Caspase-1に点変異を導入して，正常caspase-1の作用を抑制したmutantマウスを作製し，HD-transgenic miceと掛け合わせるとHD-miceに較べて運動障害の程度は軽くなり，発症や死亡も遅延したという。類似の効果はcaspaseの阻害剤であるzVAS-fmkを脳室内に持続注入することでも得られ，マウスではcaspase活性の阻害がHDの病態を緩和することが示された[27]。Caspaseの活性化は他の変性疾患や脳虚血でも報告されているが，このHD-transgenic miceにみられるcaspase1とcaspase3mRNAの上昇はminocycline投与で低下し，同時に症状の進行は遅れ生存は延長したという[3]。しかしNIの形成はminocyclineによる影響がなく，NI形成はcaspaseを介した細胞死と必ずしも相関しないのか，minocycline神経保護作用とは直接関連しないと考えられる。

b. クレアチンはミトコンドリア膜の安定化やリン酸化クレアチン濃度を高めること等を介して神経保護作用が期待される物質であり[36]，ALS-transgenic miceの病理変化を改善することが報告されており[15]，ミトコンドリア脳筋症などではヒトでの治療効果を認めたという報告もある。HD-transgenic miceの食餌中のクレアチンを増やすと脳内のクレアチン濃度も上昇し，脳や神経細胞の萎縮，NI形成が抑制され，体重減少，行動異常も改善されるという[6]。50人のHD患者に3-5gのクレアチンを計4ヶ月投与したが，臨床症状はplacebo群と差が無かったという。（Karl Kieburtz www.Huntington-Study-Group.org）

c. CoQ10（ubiquinone）はミトコンドリアのcomplex IとIIのco factorで抗酸化作用を有するが347人のHD患者でのCoQ10 300mgx2またはremacemide 200mg x3/dayのdouble blind, placebo controlled studyでは明らかな効果は無かったという[13]。

d. グルタミン酸によるexcitotoxicityの緩和

Riluzoleはglutamateのexcitotoxicityを阻害する薬剤として当初ALSの治療薬として開発された。HD-transgenic miceに投与すると，運動障害の程度には有意差が無かったが，体重の減少が抑えられ，寿命が延長したという。Riluzole100または200mg/dayをdouble-blind, placebo-controlledで63人のHD患者さんに8週間投与し不随意運

動に効果があったという[32]。Lamotorigineはグルタミン酸の放出を抑制する薬剤で抗けいれん薬として用いられている。Double-blind, placebo-controlled studyでは舞踏病様不随意運動は投与群で抑制される傾向にあったが，病像の進行は非投与群と差がなかったという[17]。Lithiumは抗躁薬として用いられるが，NMDArepeptorを介する神経細胞障害をrescueする作用が知られている。NMDA agonistであるquinolinic acidをラット線条体に注入したラットでは小型神経細胞の変性がおこりHDのモデルと考えられている。このラットにLithiumを予め投与しておくとこの変性が抑制されたという[41]。

おわりに

遺伝子異常の同定を契機にして，病態の解明が様々な方面から多角的におこなわれているだけでなく，それを動物実験で治療的に検証していくペースも非常に速くなり，その結果を踏まえたclinical trialへの移行も次々とすすんでいる。世界的にはHDに取り組む研究が最も多く，今回はHDを中心とした紹介となった。我が国ではHDの頻度は高くはないが同様の病態，治療法は他のCAG repeat病にも敷衍できる部分があることが予想され，今後積極的な治療への取り組みが期待される。

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Was the Ataxia of Pierre Marie Machado-Joseph Disease?

A Reappraisal Based on the Last Autopsy Case From la Salpêtrière Hospital

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Nosological placement of *l'hérédo-ataxie cérébelleuse de Pierre Marie* (HAC) has never been established even after several autopsy cases from the original Haudebourg family had been reported. To reappraise the clinical and pathological features of HAC in the current framework of hereditary ataxias, we screened the autopsy records of la Salpêtrière hospital and identified a patient with a diagnosis of HAC who underwent an autopsy in 1943. Clinical features included heredity compatible with autosomal dominant inheritance, spasticity, increased tendon reflexes, mask-like face, visual impairment, nuclear ophthalmoparesis, and exophthalmos in addition to progressive ataxia. Pathological lesions included the spinal cord (spinocerebellar tracts, anterolateral fascicles, and posterior column), cerebellar dentate nucleus, pontine nucleus, pallidum, motor neurons including the oculomotor nucleus, and substantia nigra. The cerebellar cortex and inferior olives were preserved. These clinical and pathological features, similar to those described in patients from the Haudebourg family, a core prototype of HAC, are indistinguishable from those of Machado-Joseph disease. It would then be possible to conclude that some of the patients historically considered to have HAC would today be classified as having Machado-Joseph disease.

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Pierre Marie, professor and head of the department of Neurology at Paris Medical School, proposed the concept of *l'hérédo-ataxie cérébelleuse* (HAC) in 1893.[1] Its precise definition, however, remains to be established,[2] because this proposal by Pierre Marie was mainly based on clinical findings with special reference to retained or exaggerated tendon reflexes[3,4] and 2 previous pathological studies,[5,6] both reporting lesions distinct from those seen in Friedreich ataxia.[7] Classification of hereditary ataxias has thereafter been confronted with difficulties because the nosological framework could not be established on a firm basis. The data presented in subsequent autopsy reports[8-11] on the affected members of the Haudebourg family, clinically described by Klippel and Durante,[3] have been considered prototypic of HAC. The current nosological position of Pierre Marie hereditary ataxia remains to be determined; one may wonder how the original cases should be classified now that the diagnosis relies on a firm genetic ground. After the last patient from the Haudebourg family was reported in 1941 by Guillain et al,[11] however, the term *HAC* has been rarely mentioned in the literature and in clinical practice.[12-15] Its nosological identity became more and more obscured and possibly has been confounded with various hereditary ataxias with retained or exaggerated tendon reflexes (other than Friedreich ataxia).

We have tried to determine to which present day diagnosis HAC could corre-

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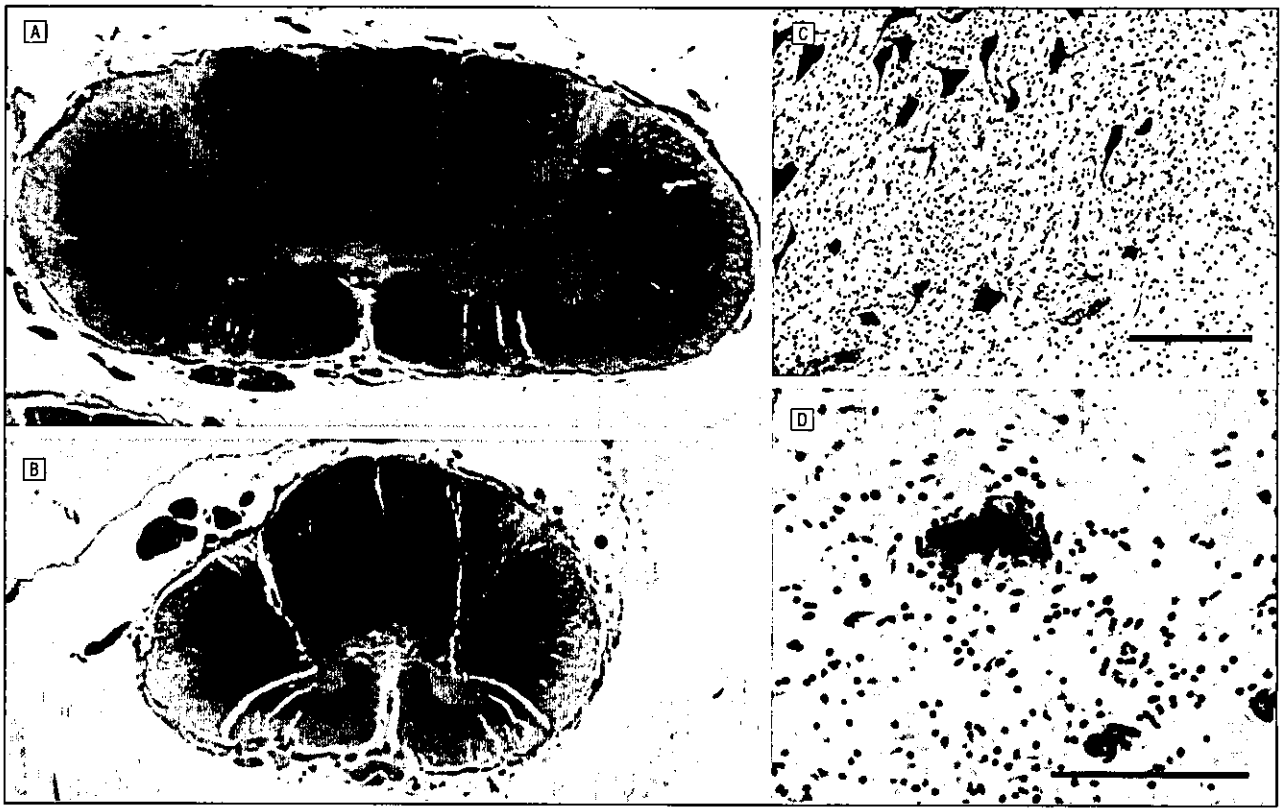

Figure 1. Present-day photographs of original slides. Macroscopic and microscopic changes of the spinal cord. A, Lower cervical cord (myelin). Anterior and posterior spinocerebellar tracts are involved bilaterally. Atrophy in the anterior horn and a slight pallor in the posterior column are present. B, Middle thoracic cord (myelin). Anterior and posterior spinocerebellar tracts are involved. The lesion extends deeper into anterolateral portion of the spinal cord. C, Anterior horn of the lumbar cord (Nissl). Massive gliosis and neuronal atrophy are observed in the anterior horn (scale bar, 200 μm). D, Clarke column (Nissl). No neuronal cells are identifiable in the Clarke column (scale bar, 100 μm).

spond. We think that this inquiry might have some utility because descendents of patients with HAC are probably still alive, receiving diagnoses and being cared for. This prompted us to screen the autopsy records of la Salpêtrière hospital to look for patients with the diagnosis of HAC. After the last report by Guillain et al,[11] only a single case was labeled with this diagnosis. Retrospective analysis of the clinicopathological features of this patient, identical to those exhibited by patients from the original Haudebourg family, revealed that the features were also compatible with those of Machado-Joseph disease (MJD).

The autopsy record (autopsy number 1541, October 15, 1943) was accompanied by 5 handwritten pages describing the clinical history and the neurological examination. This is, verbatim, their translation:

His mother presented with similar neurological features at the age of 38 with gait and speech disturbance. She died at the age of 58 at the hospice for patients with incurable diseases at Ivry. She had a daughter (of her first marriage), who was affected by the same disease.

Among children of her second marriage, two (were affected), this patient and his brother, who is 33 years old and has a difficulty in speaking. His voice is similar to that of her brother and of her sister, but he could not be examined.

This patient is admitted for:

- Difficulty in speech. Traceable to his early childhood. Slowed and slurred speech, which became progressively marked.
- Gait disturbances. They appeared at the age of 34, he swayed and hardly went up the stairs. In 1937, had to use 2 walking sticks. Numbness of the arms appeared at the same time.
- Visual difficulties: His visual acuity declined when motor deficits became apparent. He could not see well. He had the feeling of looking through a curtain. In 1938, he left his job.

Examination. Gait: He walks with difficulty. The legs are rigid and extended, gait with short steps, wide based. Movements are spastic. During walking, the head is unstable with slow swaying in anterior-posterior direction. No progression after April, 1940. Muscle tone is a little increased. Muscle strength is conserved.

Reflexes: Deep tendon reflexes are increased. Even markedly exaggerated bilaterally at patella and Achilles. Contralateral reflexes of adductors are extensive. Upper extremities: reflexes exaggerated and symmetric. Plantar reflex in flexion, defensive movement being triggered by the stimulation. Cremasteric reflexes normal at the right side, not regularly obtained at the left side. Although abdominal cutaneous reflexes are conserved only in the upper abdomen, it is weak. Clonus. Sensibility: normal.

Cerebellar signs: Marked dysmetria on four extremities. Adiadochokinesis. Speech is slowed and slurred. No intension tremor. No nystagmus. No trophic changes. No disturbances of sphincters. No extrapyramidal signs, except for expressionless face.

Cranial nerves: I: normal, II: Nerve atrophy on funduscopic examination, suggesting myopic staphyloma, III, IV, VI: bilateral paresis of the superior rectus. Other extraocular muscles are also paretic except for probably the inferior

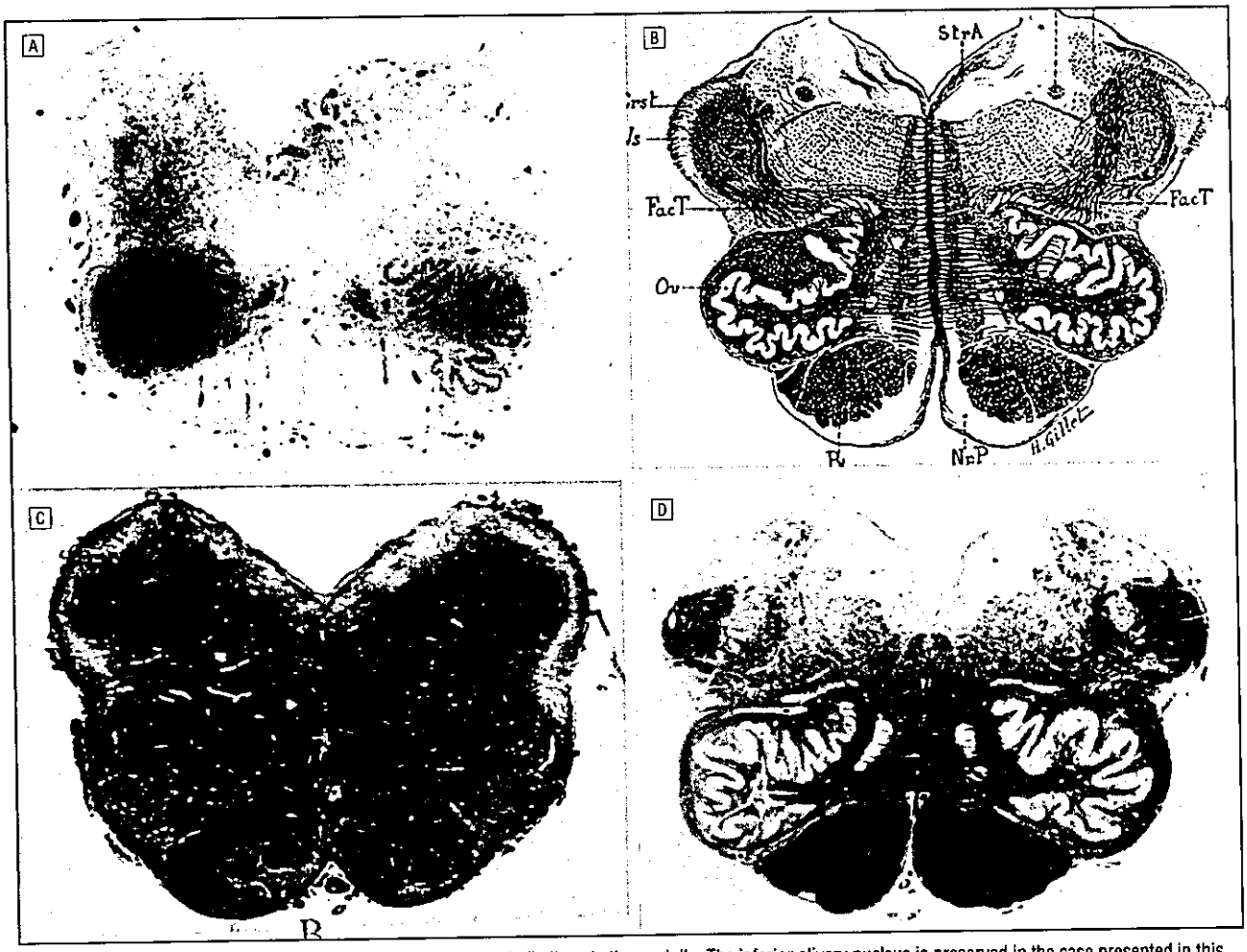

Figure 2. Present-day photographs of original slides. Macroscopic findings in the medulla. The inferior olivary nucleus is preserved in the case presented in this study (A) and patients from the Haudebourg family (B, Amélie Haudebourg, citation 10) (C, François Haudebourg, citation 9). This holds true in patients with typical Machado-Joseph disease (D).

rectus. Bilateral exophthalmos. Light reflex is detectable and very slight. VII: No facial weakness. Mask-like face. VIII: Auditory acuity normal, but, on both sides, central deficit of vestibular nerves with inexcitability. IX, X, XI, XII: Normal.

On August 3, 1943, large bilateral cavities, (on August) 15, died.

Clinical diagnosis. Hérédoataxie de Pierre Marie.

An autopsy was performed on the same day. The neuropathological report (1571), which was not signed but was probably written by Yvan Bertrand, who was in charge of the laboratory, indicated:

Marked atrophy of the brainstem, moderate atrophy of the cerebellum. Nothing particular at the level of the cerebrum. Spinal cord [**Figure 1** A and B], Very badly sampled, damaged by hammer and knife. Aspect of the spinal cord not very characteristic of hérédoataxie; sclerosis of anterolateral tracts and marginal tracts, however, can be identified [Figure 1A and B]. High cervical section: spongiosis in the dorsal column [Figure 1A].

Medulla [**Figure 2**A], damaged at autopsy. Not so atrophic relatively. Pyramids intact. Pallor of the fibers around the olives. Spongiosis of the restiform body, very atrophied. Atrophy of the solitary fascicle. Acoustic striae untouched.

Mesocephalon (=mesencephalon): Generalized atrophy, but especially remarkable in the tegmentum, where demyelination is severely advanced. On the other hand, stretching and V-shaped distortion. Medial longitudinal fasciculus: normal. Crus cerebri: normal. Sclerosis of the spinocerebellar tract is present but not massive. This moderate change is in contrast with diffuse atrophy of the brainstem without system selectivity.

Pons [**Figure 3**A and B]: equally very atrophic, pallor of the tegmentum and of several transverse pontocerebellar bundles (reduction in number rather than degeneration). Pyramidal tract untouched. Near the median line in the base of the pons, a site of prelacunar, hemorrhagic and spongy state. The cerebellar white matter is compact except in its posterior and upper portions, where subcortical rarefaction is present, a kind of prelacunar state. The dentate nucleus [Figure 3A], very atrophic, evident lesion: cell loss, liquefaction, glial infiltration and pallor of the fibers around the nucleus [Figure 3C]. This entire region is very fragile. The origin of the superior cerebellar peduncle is severely rarefied and atrophied.

Cerebellum [Figure 3D]: Laminar atrophy of moderate degree with reduction in the number of the various constituents: granule cells and Purkinje cells. As a whole, however, one has the feeling of laminar primary cortical atrophy with secondary degeneration of the centers of the brainstem.

In the cerebellum and brainstem, the atrophy is more severe than the degeneration of selected systems. The sparing of the pyramidal tract, and the spinocerebellar tract involvement authorizes the diagnosis of "hérédo-ataxie" despite the absence of ventral predominance of the lesions.

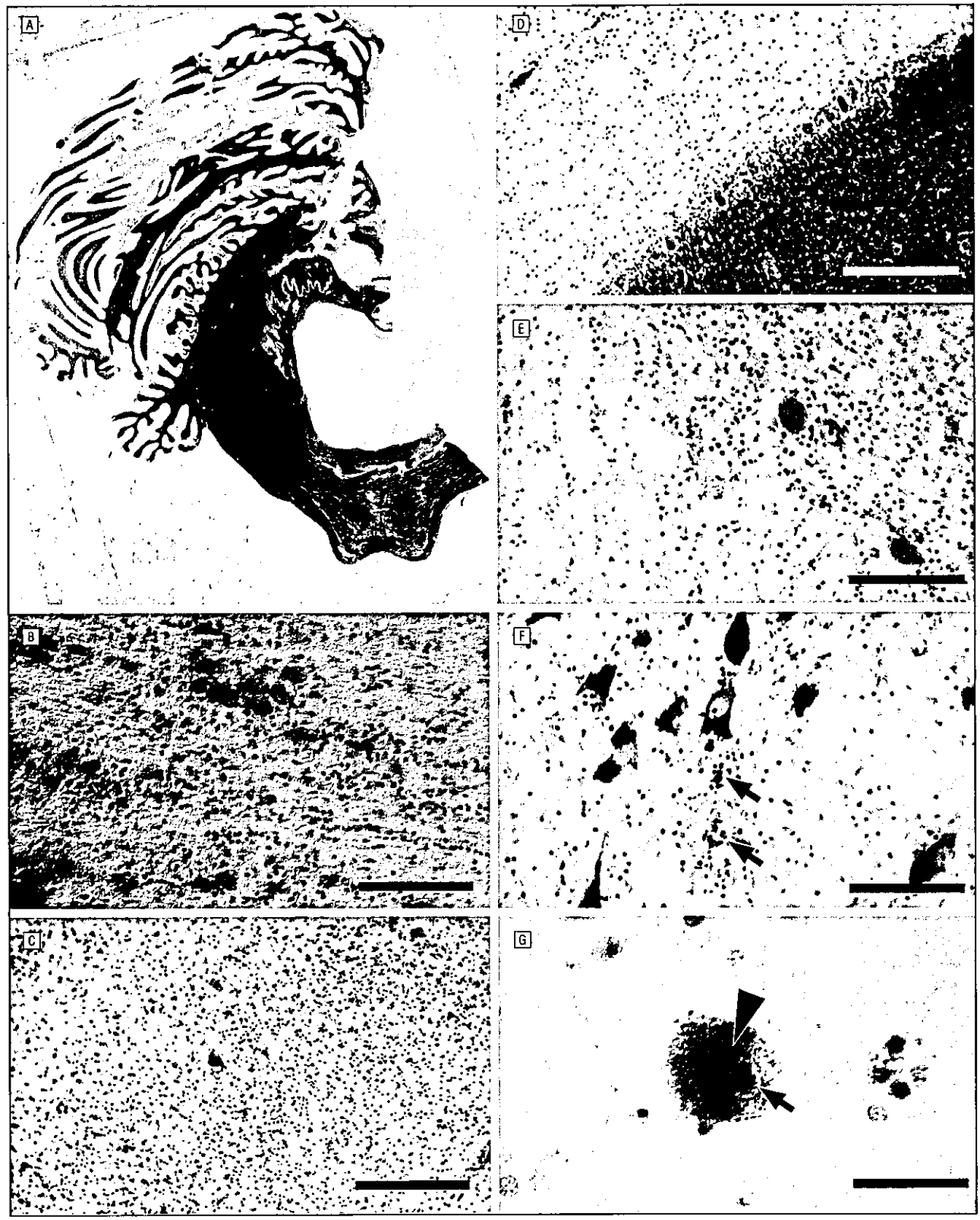

Figure 3. Present-day photographs of original slides. Macroscopic and microscopic findings in the cerebellum and basal ganglia. A, Pons and cerebellum. Despite the atrophy in the pontine base and dentate nucleus, the cerebellar cortex is relatively well preserved (myelin). B, Pontine nuclei. Gliosis and degeneration of pontine neurons (Nissl; scale bar, 50 μm). C, Dentate nucleus. Neuronal depletion and gliosis (Nissl; scale bar, 200 μm). D, Cerebellar Purkinje cells are well preserved (hematoxylin-eosin; scale bar, 200 μm) E, Oculomotor nucleus. Massive gliosis and neuronal depletion (Nissl; scale bar, 50 μm). F, Substantia nigra (pars compacta). Gliosis and free-melanin pigments (arrows) (Nissl; scale bar, 50 μm). G, Pallidum. A neuronal cell containing nucleolus (arrow) and an additional round structure (arrowhead) reminiscent of intranuclear inclusion (Nissl; scale bar, 20 μm).

Table 1. Similarity in Clinical Features Between Patients From the Haudebourg Family and Patients With Machado-Joseph Disease (MJD)

Clinical Feature	Patients From Haudenbourg Family	Autopsy Case	Patients With MJD
Heredity	AD compatible	AD compatible	AD
Ataxia	Present	Present	Present
Pyramidal signs	Present	Present	Present
Rigidity	Present	Present	Present
Mask-like face	*absence de mimique* (absence of facial expression)	*visage sans mimique* (expressionless face)	Present
Exophthalmos	*regard étonné* (astonished gaze); *yeux grand ouverts* (wide opened eyes)	Present	Stared gaze
EOM	Diplopia (nystagmus)	Nuclear paresis	Nuclear paresis
Vestibular dysfunction	Unknown	Disturbed	Caloric response, absent

Abbreviations: AD, autosomal dominant; EOM, extraocular movements.

MICROSCOPIC REEXAMINATION OF THE STAINED PREPARATIONS

Only several specimens embedded in celloidin are still available for neuropathological examination. In addition to the original description depicted earlier, the following lesions were identified: anterior horn (Figure 1C), Clarke column (Figure 1D), hypoglossal nucleus, oculomotor nucleus (Figure 3E), pars compacta of substantia nigra (Figure 3F), and pallidum. The inferior olivary nucleus was well preserved (Figure 2A). A Nomarski view of a neuron in the pallidum on a Nissl-stained section identified a possible nuclear inclusion (Figure 3G) reminiscent of those seen in most cytosine-adenine-guanine (CAG) triplet expansion diseases including MJD.[16-18]

COMMENT

Clinical features of this patient included ataxic gait with spasticity, appendicular ataxia, and slurred speech, which are shared with so-called spinocerebellar degenerations. Increased muscle tone and increased deep tendon reflexes, together with nuclear ophthalmoparesis, exophthalmos, and mask-like face were described in patients from the Haudebourg family (**Table 1**),[3,8-11,19] which reasonably led to the clinical diagnosis of HAC. These clinical features, however, have been described in some patients with the diagnosis of MJD (Table 1),[20] although the clinical features of MJD are highly variable.[21-24] Some of these features other than ataxia have also been described sometimes in patients with spinocerebellar ataxia type 1,[20,25] whereas vestibular disturbance of central origin is a distinctive feature of MJD.[26,27] Although stared gaze or bulging eye is a probable (but not exclusive) indicator of MJD,[20,25] *l'aspect étonné du facies* (astonished look of the face) or *les yeux grand ouverts* (wide, opened eyes) described in the affected members from the Haudebourg family[3] or exophthalmos in this autopsy case may be identical.

An association of pigmentary retinopathy to autosomal dominant cerebellar ataxia was considered to be a hallmark for autosomal dominant cerebellar ataxia II,[28,29] consistently found in spinocerebellar ataxia type 7.[30-32] Visual impairment due to optic atrophy, however, has recently been found to be not uncommon (13 [22.8%] of 57 cases) in a cohort of genetically confirmed cases of MJD.[33] Indeed, several patients from the Haudebourg family, as well as the first autopsied case reported as HAC,[34] also developed visual impairment. Although ultimate genetic abnormality has not yet been confirmed in the family of the autopsy case, the mode of inheritance was compatible with autosomal dominant inheritance, shared by most of the hereditary ataxias linked to CAG triplet expansion diseases, including MJD. Had trained clinical neurologists of today had a chance to examine the clincial findings of this case, they would have suspected that MJD was the most probable diagnosis even before genetic analysis.

Pathological description from this autopsy record stated that the lesions included the spinal cord (spinocerebellar tracts, anterolateral fascicles, and posterior column in the upper cervical segment), pontine nuclei, dentate nucleus, and the cerebellum as presented in **Table 2**. Involvement of the spinocerebellar tracts and anterolateral fascicles described in this autopsy case is among the principal features of HAC as described in autopsied patients from the Haudebourg family.[8-11] This is reminiscent of the morphological changes of the spinal cord in cases of MJD.[20-24,35,36] Severe involvement of the pontine nucleus with relative preservation of the cerebellar cortex, again noted in the patients from the Haudebourg family,[9,19] was recorded in this autopsy case, which is also characteristic of MJD.[20,21,23] Although this autopsy record only mentioned that the medulla oblongata was not atrophic, reviewing the slides confirmed that inferior olives were well preserved (Figure 2A), as reported in the patients from the Haudebourg family (Figure 2B and C, Table 2).[8-11,19] This is another distinguishing feature of MJD (Figure 2D) not observed in spinocerebellar ataxia type 1, type 2,[20] or type 7.[31] Relative preservation of the cerebellar cortex and inferior olives with preferential involvement of pontocerebellar and spinocerebellar projections, as seen in this autopsy case, are also shared with the autopsy cases from the Haudebourg family and individuals with MJD (Table 2). Because cerebellipetal systems wired through mossy fibers are preferentially affected, this combination could be grouped under the name *mossy-fiber type cerebellipetal degeneration*,[19] which may further include an autopsy case[37] from a large family[4] and spinopontine degeneration (SPD), initially reported by Boller and Seggara[38-40] (Table 2). It is worth mentioning that Boller and Seggara noted that clinicopathological features of SPD were similar to those described[41,42] in patients from the Haudebourg family with HAC.[8-11] Moreover, the similarity between SPD and MJD[36,43] was corroborated by the identification of SPD and MJD in the

Table 2. Pathological Features Shared by Patients From the Haudebourg Family and Patients With Machado-Joseph Disease (MJD) and Spinopontine Degeneration (SPD)

Pathological Feature	Patients From Haudebourg Family	Autopsy Case	Patients With MJD	Patients With SPD
Severity of atrophy*				
Spinocerebellar tracts	Moderate	Mild	Moderate	Mild
Clarke column	Moderate	Moderate	Severe	Moderate
Pyramidal tract	None	None	None	None
Anterolateral fascicle	Moderate	Mild	Severe	Mild
Anterior horn	Moderate	Mild	Severe	Moderate
Inferior olives	None	None	None	None
Atrophy of pons greater than cerebellum	Yes	Yes (numeric reduction)	Yes	Yes
Dentate nucleus	Moderate	Severe	Severe	None to mild
Oculomotor nuclei	Unknown	Moderate	Severe	Unknown
Substantia nigra	Mild	Moderate	Moderate	Mild
Pallidum	Unknown	Moderate (internal = external)	Moderate (internal > external)	Mild
Subthalamic nucleus	Unknown	Unknown	Moderate	Mild

*In all cases, brainstem and spinal cord atrophy were greater than cerebellar atrophy and cerebellar atrophy was greater than cerebral atrophy.

same kindred.[44] The SPD phenotype in some families was finally found to be linked to the same genetic abnormality as MJD.[45] It is therefore probable that common clinicopathological features of SPD and HAC, lumped as mossy-fiber type cerebellipetal degeneration,[19] could be applied to MJD, suggesting that these entities are to be grouped under the same diagnostic flag.

In addition to the involvement of the dentate nucleus, further examination of the histological preparations verified that the oculomotor nucleus, pars compacta of substantia nigra, and pallidum were also affected, as observed in MJD[20-24,35] and also in SPD.[38-40,43] No slides were available to examine the subthalamic nucleus. Although unstained sections are not available for immunohistochemical examination, a Nissl-stained section of the internal pallidum contained a neuron harboring a spherical structure distinct from the nucleolus (Figure 3G), which seems identical to the intranuclear inclusion seen in most CAG triplet expansion diseases including MJD.[16-18]

The proposal of Pierre Marie to isolate, from Friedreich ataxia, a group of hereditary ataxias that could be identified on the basis of retained or exaggerated tendon reflexes was really prescient. It proved to be premature, in retrospect, because it was only based on clinical signs that are known today to be present in a variety of entities that genetic tools can now distinguish. Although it is hard to define HAC in the present nosological framework of hereditary ataxias, the clinical and pathological phenotype described in patients from the Haudebourg family is one of the major prototypes of HAC. Clinical and pathological features of this autopsy case are shared with patients from the Haudebourg family, which authorized the diagnosis of HAC, as interpreted in 1943. On the other hand, retrospective review of the clinicopathological features confirmed that the phenotype of this patient is also indistinguishable from that of individuals with MJD. It seems, then, very probable that MJD reported as if it were a new disease entity might have been a description of another clinicopathological aspect of patients from the Haudebourg family with special reference to lesions in the brainstem and basal ganglia. A series of autopsy reports on the patients from the Haudebourg family mainly dealt with spinal cord lesions,[8-11,46] whereas early pathological description of MJD paid more attention to lesions in the basal ganglia and brainstem.[22-24] It is therefore probable that these 2 different aspects were considered to represent different diseases when they were, in reality, 2 different aspects of a single disease.

This autopsy case labeled with the diagnosis of HAC provided us with an opportunity to see how French neurologists and neuropathologists defined HAC in 1943 and to compare the findings with those of MJD. Although it is still debated whether HAC should be limited to a single disease entity, it seems likely that the HAC of Pierre Marie, and more specifically the Haudebourg family, includes clinicopathological characteristics indistinguishable from MJD.

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Dual enhancement of triple immunofluorescence using two antibodies from the same species

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Abstract

Triple immunofluorescence method with two mouse monoclonal antibodies and another rabbit polyclonal antibody was established with catalyzed reporter deposition (CARD) amplification on thick floating sections from the rat cerebellum. One of the monoclonal antibodies (anti-calbindin), diluted maximally, probed with anti-mouse IgG–horseradish peroxidase (HRP) and amplified with Cy5-conjugated tyramide, immunolabeled cerebellar Purkinje cells and their arborization. Subsequently, a rabbit polyclonal IgG (anti-glial fibrillary acidic protein (anti-GFAP)), probed with anti-rabbit IgG–HRP, amplified with biotin–tyramide and visualized with fluorescein-isothiocyanate (FITC)–streptavidin, immunolabeled Bergmann's glia. Another mouse monoclonal IgG (anti-SNAP25), probed with anti-mouse IgG–rhodamine without CARD amplification, selectively visualized synaptic sites, because the maximal dilution of the other monoclonal antibody (anti-calbindin) was below the detection threshold of this anti-mouse IgG–rhodamine. Separation of the two signals (calbindin and SNAP25), each detected through mouse monoclonal antibody, was then based on the difference of sensitivity either with or without CARD amplification. Triple immunofluorescence is possible when just one of the three primary antibodies is from different species. Intensification of two of the three signals provides further advantages to examine immunolocalization of multiple epitopes on histological sections.

Keywords: Catalyzed reported deposition; Multilabeling; Tyramide; Cerebellum; Development

1. Introduction

Multifluorolabeling immunohistochemistry is an indispensable tool to examine spatial relationship between epitopes on histological sections. It requires a clear separation of signals and sufficient intensity of each signal. Because the signals obtained with conventional fluorochromes conjugated with secondary antibodies are not always intense enough, several signal amplification methods have been developed. Among them, catalyzed reporter deposition (CARD) method is now frequently used as one of the methods of choice (Adams, 1992; Bobrow et al., 1989). Amplification with CARD method is mediated by horseradish peroxidase (HRP), usually conjugated with secondary antibodies and reacted with tyramide in the presence of hydrogen peroxide. This yields amplified signal represented by a reporter molecule, such as biotin or fluorochrome, conjugated in advance with tyramide, that accumulates around HRP (Adams, 1992; Bobrow et al., 1989). Signal amplification with CARD method allows further dilution of the primary antibody below the threshold detectable with non-amplified conventional method using fluorochrome-labeled secondary antibodies (Kumar et al., 1999; Speel et al., 1997; van Gijlswijk et al., 1997). Hunyaday et al. (1996) first reported that a combination of this highly sensitive method with conventional fluorolabeling enabled double immunolabeling based on this difference in the detection threshold even when the two epitopes were probed with primary antibodies of the same class from the same species. This double labeling with antibodies from the same species can be combined with an additional antibody from another species without danger of cross-reaction, as we demonstrated recently (Uchihara et al., 2003). Moreover, by using tyramide-conjugated fluorochromes, it is possible to amplify two immunofluorescent signals from two antibodies from different species (Uchihara et al., 2000). Because this dual amplification allows further dilution of these two primary antibodies, it can be theoretically combined with

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