ИНФОРМАЦИЯ ЗА:
Наименование на заболяването
Х-свързан паркинсонизъм със спастицитет синдром
Определение на заболяването
Х-свързан паркинсонизъм-спастичност синдром е с начало между 14 и 50 години, като се наблюдава по-ранно начало в последващите поколения. Някои пациенти имат само класически паркинсонов синдром (ригидност, тремор в покой и брадикинезия). Други пациенти развиват спастичност (с хиперрефлексия и положителен Бабински), последвана от развитието на паркинсонизъм. Заболяването е с бавна прогресия. Х-свързаният паркинсонизъм със спастицитет се причинява от хемизиготна мутация в ATP6AP2 гена на хромозома Xp11.
Четирицифрен код на заболяването по МКБ-10 (ако такъв е наличен)
G20
Код на заболяването по Orpha code
ORPHA363654
Епидемиологични данни за заболяването в Република България
<1 / 1 000 000; Неизвестни точни заболеваемост и болестност. Предполага се заболяваемост сходна на станалите страни в Европа.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Milanov I, Kmetska K, Karakolev B, Nedialkov E. Prevalence of Parkinson's disease in Bulgaria. Neuroepidemiology. 2001;20(3):212-4. 2. Poorkaj, P., Raskind, W. H., Leverenz, J. B., Matsushita, M., Zabetian, C. P., Samii, A., Kim, S., Gazi, N., Nutt, J. G., Wolff, J., Yearout, D., Greenup, J. L., Steinbart, E. J., Bird, T. D. A novel X-linked four-repeat tauopathy with parkinsonism and spasticity. Mov. Disord. 25: 1409-1417, 2010.
Епидемиологични данни за заболяването в Европейския съюз
<1 / 1 000 000; Неизвестни точни заболеваемост и болестност. Poorkaj и колеги (2010) съобщават за голяма фамилия с датски и немски произход, в която 5 мъже от 3 поколения са засегнати.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Poorkaj, P., Raskind, W. H., Leverenz, J. B., Matsushita, M., Zabetian, C. P., Samii, A., Kim, S., Gazi, N., Nutt, J. G., Wolff, J., Yearout, D., Greenup, J. L., Steinbart, E. J., Bird, T. D. A novel X-linked four-repeat tauopathy with parkinsonism and spasticity. Mov. Disord. 25: 1409-1417, 2010.
Оценка на съответствието на заболяването с дефиницията за рядко заболяване съгласно § 1, т. 42 от допълнителните разпоредби на Закона за здравето

Заболяването е с разпространение под 5/ 10 000 души от населението на Европейския съюз.
Критерии за диагностициране на заболяването
Диагностициране на заболяването (дефиниция на случай): Признаците и симптомите на заболяването: Х-свързан паркинсонизъм-спастичност синдром е с начало между 14 и 50 години, като се наблюдава по-ранно начало в последващите поколения. Някои пациенти имат само класически паркинсонов синдром (ригидност, тремор в покой и брадикинезия). Други пациенти развиват спастичност (с хиперрефлексия и положителен Бабински), последвана от развитието на паркинсонизъм. Заболяването е с бавна прогресия. Етиологията и патогенезата: Korvatska и колеги (2013) идентифицира промяна в c.345C-T на ATP6AP2 гена. При постмортен изследване на един засегнат пациент се установява намаление на ATP6AP2 във фронталната кора и стриатума. В допълнение се установява масивно натрупване на SQSTM1 в стриатума, което предполага нарушена автофагия и дефект в лизозомомедираната белтъчна деградация.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Poorkaj, P., Raskind, W. H., Leverenz, J. B., Matsushita, M., Zabetian, C. P., Samii, A., Kim, S., Gazi, N., Nutt, J. G., Wolff, J., Yearout, D., Greenup, J. L., Steinbart, E. J., Bird, T. D. A novel X-linked four-repeat tauopathy with parkinsonism and spasticity. Mov. Disord. 25: 1409-1417, 2010. 2. Korvatska, O., Strand, N. S., Berndt, J. D., Strovas, T., Chen, D.-H., Leverenz, J. B., Kiianitsa, K., Mata, I. F., Karakoc, E., Greenup, J. L., Bonkowski, E., Chuang, J., Moon, R. T., Eichler, E. E., Nickerson, D. A., Zabetian, C. P., Kraemer, B. C., Bird, T. D., Raskind, W. H. Altered splicing of ATP6AP2 causes X-linked parkinsonism with spasticity (XPDS). Hum. Molec. Genet. 22: 3259-3268, 2013.
Алгоритми за диагностициране на заболяването
Алгоритми за диагностициране на заболяването: Диагноза на паркинсонизма съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест. Анамнезата: Х-свързан паркинсонизъм-спастичност синдром е с начало между 14 и 50 години, като се наблюдава по-ранно начало в последващите поколения. Някои пациенти имат само класически паркинсонов синдром (ригидност, тремор в покой и брадикинезия). Други пациенти развиват спастичност (с хиперрефлексия и положителен Бабински), последвана от развитието на паркинсонизъм. Заболяването е с бавна прогресия. Диференциалната диагноза на заболяването: Други форми на паркинсонизъм с ранно начало Лабораторни, образни и хистологични изследвания: Fluoro-DOPA PET показва намалено натрупване в стриатума. Постмортем изследването показва невронална загуба в областта на субстанция нигра без натрупване на Lewy тела. В допълнение е налице известна алцхаймерподобна патология в областта на стиратума и 4-тауповторни (MAPT) –позитивни плаки. Генетични изследвания и медико-генетично консултиране: Х-свързания паркинсонизъм със спастицитет се причинява от хемизиготна мутация в ATP6AP2 гена на хромозома Xp11. Предаването на заболяването се осъществява чрез Х-свързано рецесивно унаследяване.
В т.ч. научни публикации от последните пет години и приложена

библиографска справка
1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1. 2. Poorkaj, P., Raskind, W. H., Leverenz, J. B., Matsushita, M., Zabetian, C. P., Samii, A., Kim, S., Gazi, N., Nutt, J. G., Wolff, J., Yearout, D., Greenup, J. L., Steinbart, E. J., Bird, T. D. A novel X-linked four-repeat tauopathy with parkinsonism and spasticity. Mov. Disord. 25: 1409-1417, 2010. 3. Korvatska, O., Strand, N. S., Berndt, J. D., Strovas, T., Chen, D.-H., Leverenz, J. B., Kiianitsa, K., Mata, I. F., Karakoc, E., Greenup, J. L., Bonkowski, E., Chuang, J., Moon, R. T., Eichler, E. E., Nickerson, D. A., Zabetian, C. P., Kraemer, B. C., Bird, T. D., Raskind, W. H. Altered splicing of ATP6AP2 causes X-linked parkinsonism with spasticity (XPDS). Hum. Molec. Genet. 22: 3259-3268, 2013.
Алгоритми за лечение на заболяването
Алгоритми за лечение на заболяването: Лечение на паркинсонизма съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест. Терапевтичните подходи към заболяването, в това число консервативни и оперативни, техните предимства, рискове и очаквана ефективност: Има съобщение за един пациент със заболяването с слаб отговор на Леводопа терапия и последващо развитие на дистонни дискинезии.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1. 2. Poorkaj, P., Raskind, W. H., Leverenz, J. B., Matsushita, M., Zabetian, C. P., Samii, A., Kim, S., Gazi, N., Nutt, J. G., Wolff, J., Yearout, D., Greenup, J. L., Steinbart, E. J., Bird, T. D. A novel X-linked four-repeat tauopathy with parkinsonism and spasticity. Mov. Disord. 25: 1409-1417, 2010.
Алгоритми за проследяване на заболяването
Алгоритми за проследяване на заболяването: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест. Възможни усложнения: Развитие на леводопа-индуцирани дистони дискинезии.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1. 2. Poorkaj, P., Raskind, W. H., Leverenz, J. B., Matsushita, M., Zabetian, C. P., Samii, A., Kim, S., Gazi, N., Nutt, J. G., Wolff, J., Yearout, D., Greenup, J. L., Steinbart, E. J., Bird, T. D. A novel X-linked four-repeat tauopathy with parkinsonism and spasticity. Mov. Disord. 25: 1409-1417, 2010.
Алгоритми за рехабилитация на заболяването
Алгоритми за рехабилитация на заболяването: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Национален консенсус за диагностика и лечение на Паркинсоновата болест, Двигателни заболявания, 2013, 10, 1.
Необходими дейности за профилактика на заболяването (ако такива са

приложими)

Дейности за профилактика на заболяването:
Първична, вторична и третична превенция: Х-свързаният паркинсонизъм със спастицитет се причинява от хемизиготна мутация в ATP6AP2 гена на хромозома Xp11. Предаването на заболяването се осъществява чрез Х-свързано рецесивно унаследяване. При семейства с установена мутация би могло да се обсъжда пренатална диагностика.

В т.ч. научни публикации от последните пет години и приложена библиографска справка

1. Poorkaj, P., Raskind, W. H., Leverenz, J. B., Matsushita, M., Zabetian, C. P., Samii, A., Kim, S., Gazi, N., Nutt, J. G., Wolff, J., Yearout, D., Greenup, J. L., Steinbart, E. J., Bird, T. D. A novel X-linked four-repeat tauopathy with parkinsonism and spasticity. Mov. Disord. 25: 1409-1417, 2010.
2. Korvatska, O., Strand, N. S., Berndt, J. D., Strovas, T., Chen, D.-H., Leverenz, J. B., Kiianitsa, K., Mata, I. F., Karakoc, E., Greenup, J. L., Bonkowski, E., Chuang, J., Moon, R. T., Eichler, E. E., Nickerson, D. A., Zabetian, C. P., Kraemer, B. C., Bird, T. D., Raskind, W. H. Altered splicing of ATP6AP2 causes X-linked parkinsonism with spasticity (XPDS). Hum. Molec. Genet. 22: 3259-3268, 2013.

Предложения за организация на медицинското обслужване на пациентите и за финансиране на съответните дейности, съобразени с действащата в страната нормативна уредба

Създаването на Национален експертен център „Редки невродегенеративни заболявания, протичащи с когнитивни, поведенчески и моторни нарушения" за диагностика, лечение и проследяване и рехабилитация включително и на пациенти с това заболявания под ръководството на чл.кор.проф.д-р Л. Трайков, дмн (национален експерт с най-голям опит и принос за диагностиката и лечението на тези заболявания).

Описание на опита с конкретни пациенти със съответното рядко заболяване (ако има такъв)

Опитът на кандидатстващия експертен център под ръководството на чл. кор. проф.Трайков за диагноза и лечение на редки заболявания, протичащи с паркинсонизъм с и без когнитивни нарушения, датира от 2001 година със създаването на център за диагноза и лечение на невродегенеративни заболявания, протичащи с деменция и допълнително на център за диагноза и лечение на Паркинсонова болест. От дълги години този център е реферирал център за заболявания, протичащи с паркинсонизъм с и без когнитивни нарушения, особено за комплексни, редки и наследствени случаи. През годините вследствие на натрупания опит и труд, както и значителен брой на пациенти с тези редки заболявания, реферирани към центъра са осъществени няколко дисертации в областта: 1. Когнитивни нарушения при Паркинсонова болест (защитена дисертация за доктор по медицина от д-р Мария Петрова, 2010 г., ръководител: чл.-кор. проф. Лъчезар Трайков), 2. Лонгитудинално проследяване на когнитивните нарушения при Паркинсонова болест (защитена дисертация за доктор по медицина от д-р Явор Желев, 2012 г., ръководител: чл.-кор. проф. Лъчезар Трайков) и 3. Клинико-генетични корелации при невродегенеративни заболявания, протичащи с паркинсонизъм (защитена дисертация за доктор по медицина от д-р Радка Павлова, 2013 г., ръководител: чл.-кор. проф. Лъчезар Трайков). Събрана е база данни за отделни пациенти с отделни групи редки заболявания, протичащи с паркинсонизъм с и без когнитивен дефицит с подробно

фенотипизиране на всеки един случай, което дава възможност за добър мониторинг на пациентите, както и изследователски анализ върху характеристиката на отделните заболявания. Дейността на центъра по отношение на диагноза и лечение на редки заболявания, протичащи с моторни и когнитивни нарушения, обхваща всички диагностични дейности съобразно новите диагностични критерии на тези заболявания, включително допълнителни изследвания, които са нужни за диференциална диагноза на атипични/ранни/наследствени случаи, включващи изследвания за биомаркери, невроизобразяващи и генетични фактори.

Публикации:

1. Pavlova R, Mehrabian S, Petrova M, Skelina S, Mihova K, Jordanova A, Mitev V, Traykov L. Cognitive, neuropsychiatric, and motor features associated with apolipoprotein E ε4 allele in a sample of Bulgarian patients with late-onset Parkinson's disease. Am J Alzheimers Dis Other Demen. 2014 Nov;29(7):614-9.
2. Petrova M, Raycheva M, Traykov L. Cognitive profile of the earliest stage of dementia in Parkinson's disease. Am J Alzheimers Dis Other Demen. 2012 Dec;27(8):614-9.
3. Petrova M, Raycheva M, Zhelev Y, Traykov L. Executive functions deficit in Parkinson's disease with amnestic mild cognitive impairment. Am J Alzheimers Dis Other Demen. 2010 Aug;25(5):455-60.
4. Kochev D, Petrova J, Petrova M, Krastev D, Traykov L. Possibility of combined assessment of biomarkers in early Parkinson's disease. International Journal of Science and Research, 2014, 3, 10, 1332-1334;
5. Петрова М., Райчева М., Пенев Л., Григорова О., Желев Я., Трайков Л. Когнитивни различия между леко когнитивно нарушение и деменция при Паркинсонова болест. Българска Неврология, 2010, 4, 168-172.
6. Петрова М., Райчева М., Мехрабиан Ш., Желев Я., Ангов Г. Трайков Л. Връзки между депресията и когнитивните дефицити при пациенти с Паркинсонова болест и леко когнитивно нарушение. Българска Неврология, 2010, 10, 3, 122-125.
7. Петрова М., Трайков Л. Рискови фактори за развитие на когнитивни нарушения и деменция при Паркинсонова болест. Българска Неврология, 2010, 10, 3, 98-102.
8. Петрова М., Райчева М., Трайков Л. Връзки между предоминантния моторен подтип и когнитивни дефицити при пациенти с Паркинсонова болест с леко когнитивно нарушения. Българска Неврология, 2010, 4, 161-164.
9. Петрова М., Трайков Л. Особености в профила и диагностика на когнитивните нарушения при Паркинсонова болест, Неврология и Психиатрия, 2011, 1, 43.
10. Павлова Р, Мехрабиан Ш, Скелина С, Желев Я, Михова К, Кънева Р, Митев В, Йорданова А, Трайков Л. Характеристика на дегенеративния паркинсонов синдром в зависимост от Аполипопротеин Е генотипа. Неврология и психиатрия, 4, 30-33, 2014;
11. Петрова М., Григорова О, Желев Я., Павлова Р., Владимиров Б., Трайков Л. Влияние на Дуодопа върху моторните и немоторите усложнения при

напреднала Паркинсонова болест. МЕДИКАРТ: Неврология и Психиатрия, 2014, 1, 24-29.
12. Кочев Д., Петрова Ю., Петрова М., Трайков Л. Оценка на ехогенността на субстанция нигра при пациенти с ранна Паркинсонова болест. Медицински Преглед, 2014, 50, 5, 45-47.
13. R. Pavlova, K. Mihova, S. Mehrabian, M. Petrova, S. Skelina, R. Kaneva, V. Mitev, L. Traykov. Novel LRRK2 6165C>G mutation in a patient with Parkinson's disease-dementia: a case report. In: JOINT CONGRESS OF EUROPEAN NEUROLOGY Istanbul, Turkey, May 31-June 3, 2014.
14. Pavlova R., K. Mihova, S. Mehrabian, M. Petrova, S. Skelina, R. Kaneva, A. Jordanova, V. Mitev, L. TraykovLRRK2 mutation c.4536+3A>G in a patient with multiple system atrophy: a case report. In: In: JOINT CONGRESS OF EUROPEAN NEUROLOGY Istanbul, Turkey, May 31-June 3, 2014.

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A Novel X-linked 4-Repeat Tauopathy with Parkinsonism and Spasticity

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Abstract

The parkinsonian syndromes comprise a highly heterogeneous group of disorders. Although 15 loci are linked to predominantly familial Parkinson's disease (PD), additional PD loci are likely to exist. We recently identified a multi-generational family of Danish and German descent in which five males in three generations presented with a unique syndrome characterized by parkinsonian features and variably penetrant spasticity for which X-linked disease transmission was strongly suggested (XPDS). Autopsy in one individual failed to reveal synucleinopathy; however, there was a significant 4-repeat tauopathy in the striatum. Our objective was to identify the locus responsible for this unique parkinsonian disorder. Members of the XPDS family were genotyped for markers spanning the X chromosome. Two-point and multipoint linkage analyses were performed and the candidate region refined by analyzing additional markers. A multipoint LOD_{max} score of 2.068 was obtained between markers DXS991 and DXS993. Haplotype examination revealed an approximately 20 cM region bounded by markers DXS8042 and DXS1216 that segregated with disease in all affected males and obligate carrier females and was not carried by unaffected at-risk males. To reduce the possibility of a false positive linkage result, multiple loci and genes associated with other parkinsonian or spasticity syndromes were excluded. In conclusion, we have identified a unique X-linked parkinsonian syndrome with variable

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The authors report no conflicts of interest.

Author's Roles.

1. Research project: A. Conception, B. Organization, C. Execution;
2. Familial Evaluations: A. Clinical, B. Pathological;
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spasticity and 4-repeat tau pathology, and defined a novel candidate gene locus spanning approximately 28 Mb from Xp11.2-Xq13.3.

Keywords

Genetic linkage; Parkinson's disease/parkinsonism; X-linked parkinsonism; X-linked spastic paraparesis; tauopathy

Introduction

The parkinsonian syndromes comprise a heterogeneous group of disorders characterized clinically by postural instability, rigidity, bradykinesia and resting tremor. Pathological features of classic Parkinson's disease (PD) include selective neurodegeneration of dopaminergic neurons in the *substantia nigra* and cytoplasmic Lewy bodies (LBs). Other parkinsonian diseases, such as progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) have predominant tau pathology. Mutations have been identified in a number of PD genes [1], and susceptibility loci [2–8] and potentially protective alleles have been reported [9, 10]. Other parkinsonian loci are likely to exist.

Linkage regions or genetic variation on the X-chromosome associated with late-onset PD (Xq21-25 and Xp11.23) [4,11] or with neurodegenerative disorders that present with parkinsonian features (DYT3, Xq13 and FXTAS, Xq27.3)[12,13], have been reported. We identified a multi-generational family with a unique syndrome characterized by parkinsonism and variably penetrant spasticity in which X-linked disease transmission was strongly suggested (XPDS). We conducted whole-X-chromosome linkage studies to determine if the X-chromosome susceptibility locus overlaps with existing candidate regions or if a novel X-linked candidate region is responsible for XPDS.

Subjects and Methods

Subjects

Members from a four generation American family of Danish and German ethnic descent with a unique parkinsonian syndrome were evaluated at the Medical Genetics Clinic at the University of Washington (UW) Medical Center or the Neurology Clinics at the Puget Sound Veterans Affairs Hospital (T.D.B. or A.S.) and Oregon Health and Sciences University (J.N.) (Figure 1). Subjects gave informed consent for blood draw and DNA studies (protocols approved by the institutional review boards). Blood samples were obtained from nine family members, including all affected living males.

Neuropathology

Standard gross and microscopic neuropathologic evaluation was performed on the only autopsy case. Histologic stains included hematoxylin and eosin, Luxol Fast Blue, Bielschowsky, and Gallyas. Immunohistologic evaluation used the following antibodies: Ab peptide (6E10, Signet Labs, 1:400), alpha-synuclein (LB509, 1:1000 and syn 303, 1:500, gifts J.Q. Trojanowski), glial fibrillary acidic protein (polyclonal GFAP, Dako, 1:1000), tau (AT8, Endogen, 1:250; PHF-1, gift P. Davies, 1:10; RD3 and RD4, Upstate Cell Signaling Solutions, 1:800 and 1:80 respectively; Tau-2, Sigma, 1:500), SMI-31 (Sternberger Monoclonals, 1:4000), TDP43 (Protein Tech, 1:2000), and ubiquitin (Dako, 1:150).

Genotyping

DNA extracted from leukocytes or B-lymphoblastoid cell lines was used to amplify eighteen microsatellite markers spanning the X chromosome (panel 28 ABI-PRISM Linkage Mapping Set v 2.0, Perkin Elmer) at an average spacing of 10 cM. Alleles were resolved on an ABI PRISM 310 genetic analyzer running GENESCAN 2.1 software and analyzed with GenotyperR (v 2.5). To refine the candidate region, five additional Marshfield genetic markers (Xp: DXS8016, DXS8042 and Xq: DXS1217, DXS1196, DXS1216; Research Genetics Huntsville, AL) were genotyped and haplotypes were constructed [14]. Exclusion mapping of autosomal dominant familial spastic paraplegias (SPG) 3A and 4 and *FBXO7*, responsible for a parkinsonian-pyramidal syndrome [15] were performed and analyzed as above.

Statistical analysis

Power analysis was performed with SLINK, a modified program of the LINKAGE package (v. 5.1) [16, 17]. Two-point, multipoint, and haplotype analyses were performed with GENEHUNTER-IMPRINTING (v.1.3) [18, 19]. Allele frequencies were obtained from CEPH. Sex-averaged map distances are available from Marshfield (http://research.marshfieldclinic.org/genetics/)[20].

Sequencing of XPDS candidate genes

XPDS candidate genes in the minimal linkage region were prioritized on the basis of expression patterns, function, or similarity to genes involved in neurodegenerative disorders, and sequenced using DNA from one affected male and one unaffected female carrier (IV-5 and III-12, Figure 1). SNPs were confirmed in affected male II-9 and female carrier III-2 by DNA sequencing. PCR primer pairs were designed to amplify candidate gene exons with at least 100 nt of flanking intronic sequence [21]. PCR products were treated with ExoSAP-IT (USB Biochemical) and sequenced with dye-terminator cycle technology (Big-Dye v. 3.1) on an ABI310 DNA analyzer.

Genes for known parkinsonian syndromes not in the region of the linkage signal were also evaluated. Sequencing of *MAPT*, *PARK2*, *LRRK2* and *SNCA1* exons and gene dosage analysis of *PARK2* exons were performed in two affected males and two unaffected obligate carrier females [21, 22]. The DSC3 single nucleotide polymorphism (SNP) associated with X-linked parkinsonism-dystonia (*DYT3*) was also sequenced [23].

Results

XPDS phenotype

The XPDS disease phenotype presents in the second through fifth decades with an onset range from 14 to 50 years. Age of disease onset varied between generations, with earlier ages of presentation in the most recent generation. Two persons had only a parkinsonian syndrome. Two and possibly three others first showed spasticity, followed by parkinsonian features. The disease followed a slowly progressive course with increasing resting tremor. Affected males in the first generation lived a normal life span.

Clinical characteristics of five affected males are summarized in Table 1. Two brothers (II-2 and II-9) had onset in their 50s of a typical parkinsonian syndrome consisting of cogwheel rigidity, resting tremor, and bradykinesia; neither developed spasticity. Rigidity and resting tremor were mildly asymmetrical in individual II-9 and moderately asymmetrical in individual II-2. Individual II-6, with the non-disease associated haplotype, died at age 82 and was clinically unaffected.

Individual III-8 gave a history that spasticity developed in his early 20s. He had the onset of a parkinsonian syndrome at age 44 with resting tremor, rigidity, and bradykinesia and reported a mild response to l-dopa. He had signs of spasticity with hyperactive tendon reflexes and Babinski responses. An MRI showed mild diffuse atrophy and disproportionate enlargement of lateral and 3rd ventricles compared to the extra-axial CSF spaces.

Individual IV-5 had the onset of spastic paraparesis at age 14. He subsequently developed a parkinsonian syndrome that included resting tremor and bradykinesia. At age 30 a fluoro-DOPA PET scan showed bilateral decreased uptake in the putamen (R>L) with normal uptake in the caudate consistent with PD. He showed a mild response to l-dopa but with prominent dystonic dyskinesias. At age 31 bilateral electrodes were placed in the subthalamic nucleus for deep brain stimulation, which improved motor symptoms, including tremor. A few months later a series of generalized convulsions was controlled with mysoline and have not recurred. He tested negative for mutations in SCA1, 2, 3, 6, 7, and 8 and had normal long-chain fatty acids.

Individual IV-1 had the onset of marked spastic paraparesis at approximately age 26. At age 30 he had a stiff scissoring gait, hyperactive tendon reflexes, ankle clonus, cogwheel rigidity in his arms and resting tremor of the right hand. At age 36, parkinsonian features were more prominent including masked facies, asymmetric resting tremor of hands and feet, and cogwheel in his upper limbs. He had no bradykinesia or retropulsion. He now had bilateral Babinski reflexes as well as the previously noted signs of spasticity.

Neuropathology of XPDS

Neuropathology was performed on individual II-2 who died at age 86. Gross examination revealed a brain weight of 1235 g with mild ventriculomegaly. Microscopic examination revealed neurofibrillary tangle (NFT) pathology limited to the medial temporal lobe, consistent with a Braak stage of III [24]. NFTs and neurites in the medial temporal lobe were immunopositive for all tau antibodies including RD3 and RD4 (Figure 3). Diffuse Aβ deposits were observed in the neocortex and limbic system, however neuritic plaques were only seen in the medial temporal lobe. The severity of plaques and tangle pathology was not sufficient to fulfill criteria for Alzheimer's disease (AD) [25]. There was mild to moderate neuronal loss in the *substantia nigra* with rare NFT pathology and no LBs. Evaluation for β-synuclein failed to reveal pathologic lesions in the brainstem, limbic system, basal ganglia, or neocortex. TDP-43 immunostaining was negative.

Anterior portions of the striatum, including both putamen and caudate, demonstrated plaque-like structures that were Gallyas, AT8, PHF-1, and RD4 immunopositive, while RD3 and ubiquitin immunonegative. Notably, in the same case, the limited AD changes observed in the medial temporal lobe were immunopositive with RD3 (Figure 3H). Double immunolabeling with GFAP and AT8 revealed a close association of astrocytes and tau positive processes (Figure 3E), and individual glial cells had AT8 immunopositive processes (Figure 3F). Similar plaque-like structures were not observed in other regions including the neocortex and limbic system. SMI-31 (anti-neurofilament) immunostaining failed to reveal ballooned neurons in the amygdala, hippocampus, basal ganglia, and neocortex.

XPDS linkage analysis

Disease inheritance is consistent with X-linked transmission (Figure 1). All five affected males in three generations are related through unaffected females and none of the three sons of two affected males are affected. Assuming a disease frequency of 0.00001, 90% penetrance, and four alleles of equal frequency, a simulation study estimated a maximum two-point LOD score of 1.81 at recombination fraction, θ, of 0.00. Two-point analysis

obtained a maximum LOD score of 1.46 at DXS993. Multipoint analyses using 18 markers identified a candidate region delineated by DXS1214 (Xp sex averaged location [SAL] 33.54 cM) and DXS990 (Xq SAL 60.62 cM), and provided a maximum LOD score of 2.02. Inclusion of five additional markers narrowed the critical region to ~20 cM defined by DXS8042 (Xp SAL 37.87 cM; Xp11.2) and DXS1216 (Xq SAL 53.58 cM; Xq13.3). The XPDS disease haplotype was not present in the unaffected at-risk male (II-6). A final maximum LOD score of 2.068 was obtained between markers DXS991 and DXS993 (Figure 2). For an X-linked disorder, a LOD score >2 provides significant evidence in favor of linkage, with LOD~2 corresponding to p~.05.

XPDS critical region

Human genomic sequence for the DXS8042-DXS1216 critical region is available as five contigs (NT_079573, NT_086939, NT_011638, NT_011630, and NT_011669) spanning ~28.5 Mb that includes an ~3 Mb gap encompassing the pericentromeric region. A comprehensive gene retrieval in this region (UCSC Browser March 2006 and NCBI Map Viewer Build 36.1) identified ~370 RefSeq genes, including multiple splice variants per gene. With splice variants represented as a single gene ~200 candidate genes remain, including 29 known disease loci and the G-, melanoma-, and P-antigen-family clusters and a synovial sarcoma breakpoint clusters. In addition, 118 hypothetical genes, twelve microRNAs, and one small nuclear RNA are annotated in the critical region. The observation of earlier onset in more recent generations, one feature of anticipation, raises the possibility of a nucleotide repeat expansion mechanism for XPDS. The XPDS candidate region harbors 174 simple trinucleotide repeats (range 8-90.7 repeat units) where 36 repeats are found in genes (31 intronic, 3 in UTRs and in one exon each in *SHROOM4* and *RBM10*). Disruptions in *SHROOM4* are linked to Stocco dos Santos X-linked mental retardation syndrome. *RBM10* contains an imperfect GGA repeat in exon 3 that is not expanded in individuals III-12 and IV-5 (Figure 1) and no other coding sequence change was detected.

Fifty-eight other candidate genes within the XPDS critical region were excluded due to lack of expression in the brain or classification as disease genes for distinctly different disorders. Seventeen candidate genes were selected on the basis of central nervous system expression and other characteristics (Table 2). Proteins that function within the ubiquitin/proteosome, mitochondrial, or oxidative stress pathways were given high priority. All exons and splice junctions of these genes were sequenced. Polymorphisms in dbSNP were identified in *UBA1* (rs11558783), *USP11* (rs10126669), *SYN* (rs2294219), and *TIMM17B* (rs1128363). A novel A→G nucleotide change encoding an R38Q amino acid substitution in *GRIPAP1* was identified in one carrier female and was not present in either affected male. No other non-polymorphic changes were found.

Candidate gene analysis outside the linkage region

Although a LOD score of 2.068, the maximum possible for this family's structure, provides approximately 95% confidence for the X-chromosome locus we identified, a 5% possibility remains that the disease gene lies elsewhere. Given that the sequence analyses of positional candidates had not yielded a causative mutation, we believed it to be prudent to rule out known genes that cause similar parkinsonian phenotypes. The disease-single-nucleotide change 3 (DSC3) in exon 4 of the multiple transcript system that is diagnostic for X-linked dystonia-parkinsonism (*DYT3*) was ruled out by targeted analysis in affected male IV-5. Several autosomal genetic risk factors for parkinsonism were evaluated by DNA sequencing of the same subject: *PARK2, MAPT, SNCA,* and *LRRK2* [26]. No pathogenic mutations were identified in these genes. No dosage variations were identified in *PARK2. MAPT* SNP analysis revealed that affected subjects III-8 and IV-5 had H1/H2 haplotypes, while the

remaining family members had H1/H1 haplotypes. Linkage to *FBXO7* and *PLA2G6* (22q12-q13), atlastin (SPG3, 14q11) and spastin (SPG4, 2p22) were excluded.

Discussion

We have described a unique parkinsonian syndrome, XPDS, mapping to Xp11.2-Xq13.3. Affected males present with a neurologic disease in which everyone eventually develops parkinsonian features with variable spasticity. XPDS is unlike idiopathic PD because of prominent spasticity and a lack of LB pathology and is unlike classic hereditary spastic paraplegia because of the co-occurrence of parkinsonian features.

We identified an ~20 cM region on the X-chromosome of shared genotypes among affected males, providing significant evidence for linkage (p .05). It is an unlikely alternative that an autosomal locus is responsible for the disease (with reduced penetrance in females) or that the more classic PD cases are disease phenocopies given the transmission pattern in the family of affected males in three generations related through unaffected females and the absence of mutations in genes responsible for three common autosomal parkinsonian disorders. Detailed analyses that ruled out the potential involvement of nine autosomal PD-associated genes strengthens our confidence in the X-chromosome linkage findings; however, until the X-linked disease gene and mutation are identified the possibility of a false positive linkage finding remains.

The XPDS locus does not overlap with any known X-chromosome PD or spasticity locus. Pelizaeus-Merzbacher disease, X-linked adrenoleukodystrophy, the XDP locus [23, 27], Fragile-X tremor/ataxia syndrome [11] and PARK12 [28] all map distal to XPDS, Thus, XPDS is a novel parkinsonian syndrome with a unique gene locus.

Pathological findings in the one autopsied case failed to reveal β-synuclein immunopositive inclusions or neurites that are generally observed in PD. However, there were plaque-like structures observed in the striatum that were positive with silver stain, and tau and GFAP immunopositive. These aggregates of tau positive glial processes had the appearance of astrocytic plaques, a frequent finding in CBD [29]. Consistent with CBD the astrocytic plaque processes were immunopositive with the 4-repeat (4R), but not the 3-repeat (3R), tau antibody suggesting this was a 4R tauopathy. Unlike CBD, we did not observe astrocytic plaques in other brain regions, nor did we observe CBD-associated pathologic changes such as ballooned neurons, neuropil threads in white matter, or oligodendroglial tau pathology. Thus, neuropathologically this appears to be a distinct 4R tauopathy. Similar to CBD and PSP, there was a predominance of the H1 haplotype in this family [30].

Two autosomal recessive syndromes share features of both parkinsonism and pyramidal track signs. These are Kufor-Rakeb and a parkinsonian-pyramidal syndrome with mutations in *ATP13A2* and *FBX07*, respectively [31, 32]. In addition to different inheritance patterns these disorders have earlier onset and more severe findings than in the present family. They may represent the disorder described by Davison (1954) with one autopsy showing nerve cell loss in the *globus pallidus* and *substantia nigra* without LBs and pallor of the pyramidal tracks [33].

The XPDS critical region contains ~200 candidate genes and 118 hypothetical genes. We selected 17 candidate genes for sequencing, including a ubiquitin-related gene (*UBQLN*), two mitochondrial-related genes (*NDUFB11* and *TIMM17B*), two protein kinases (*CASK* and *PCTK1*), and two G-protein coupled receptors (*GPR34* and *GPR173*). The remaining candidate genes were selected for high CNS expression levels. Within the time frame of this study five candidate genes were published as causing distinctly different diseases (*CASK*, *UBA1*, *PORCN*, *SYN*, and *EFNB1*; Table 2). No disease-associated mutations or

polymorphisms were identified in these genes. Screening of seven additional genes, based on CNS expression is underway including *CRSP2* (cofactor for SP1 transcriptional activation), *DDX3X* (putative RNA helicase), *RGN* (senescence marker protein), *RBM10* (RNA binding motif protein), *USP11* (ubiquitin specific protease), *PPP1R3F* (protein phosphatase inhibitory subunit), and *MAOB*, because of previous linkage studies suggesting it is a PD susceptibility locus [34]. Although brain-specific micro-RNAs have been implicated in neurodegeneration [35], the micro-RNAs that map to the critical region are implicated in erythropoietic, immunologic, and oncogenic pathways and are unlikely to cause XPDS. Our analyses have only ruled out exon deletions for those exons that demonstrated SNP heterozygosity. Array comparative genomic hybridizations may be used to exclude copy number variants and additional screening for triplet repeat expansions and PD-associate SNPs (genome wide association studies) may be employed. Ascertainment of additional XPDS family members may further refine the critical region and aid in identifying the disease gene.

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References

1. Yang YX, Wood NW, Latchman DS. Molecular basis of Parkinson's disease. Neuroreport. 2009; 20(2):150–156. [PubMed: 19151598]
2. Gasser T, Muller-Myhsok B, Wszolek ZK, et al. A susceptibility locus for Parkinson's disease maps to chromosome 2p13. Nat Genet. 1998; 18(3):262–265. [PubMed: 9500549]

3. Li YJ, Scott WK, Hedges DJ, et al. Age at onset in two common neurodegenerative diseases is genetically controlled. American Journal of Human Genetics. 2002; 70(4):985–993. [PubMed: 11875758]
4. Pankratz N, Nichols WC, Uniacke SK, et al. Genome-wide linkage analysis and evidence of gene-by-gene interactions in a sample of 362 multiplex Parkinson disease families. Hum Mol Genet. 2003; 12(20):2599–2608. [PubMed: 12925570]
5. Xu PY, Liang R, Jankovic J, et al. Association of homozygous 7048G7049 variant in the intron six of Nurr1 gene with Parkinson's disease. Neurology. 2002; 58(6):881–884. [PubMed: 11914402]
6. Martin ER, Scott WK, Nance MA, et al. Association of single-nucleotide polymorphisms of the tau gene with late-onset Parkinson disease. JAMA. 2001; 286:2245–2250. [PubMed: 11710889]
7. Hattori N, Yoshino H, Tanaka M, Suzuki H, Mizuno Y. Genotype in the 24-kDa subunit gene (NDUFV2) of mitochondrial complex I and susceptibility to Parkinson disease. Genomics. 1998; 49(1):52–58. [PubMed: 9570948]
8. Buervenich S, Carmine A, Galter D, et al. A rare truncating mutation in ADH1C (G78Stop) shows significant association with Parkinson disease in a large international sample. Arch Neurol. 2005; 62(1):74–78. [PubMed: 15642852]
9. Juyal RC, Das M, Punia S, et al. Genetic susceptibility to Parkinson's disease among South and North Indians: I. Role of polymorphisms in dopamine receptor and transporter genes and association of DRD4 120-bp duplication marker. Neurogenetics. 2006; 7(4):223–229. [PubMed: 16816977]
10. Zabetian CP, Anderson GM, Buxbaum SG, et al. A quantitative-trait analysis of human plasma-dopamine beta-hydroxylase activity: evidence for a major functional polymorphism at the DBH locus. Am J Hum Genet. 2001; 68(2):515–522. [PubMed: 11170900]
11. Hagerman RJ, Leehey M, Heinrichs W, et al. Intention tremor, parkinsonism, and generalized brain atrophy in male carriers of fragile X. Neurology. 2001; 57(1):127–130. [PubMed: 11445641]
12. Kupke KG, Graeber MB, Muller U. Dystonia-parkinsonism syndrome (XDP) locus: flanking markers in Xq12-q21.1. Am J Hum Genet. 1992; 50(4):808–815. [PubMed: 1550125]
13. Hagerman RJ, Hagerman PJ. Fragile X syndrome: A model of gene-brain-behavior relationships. Molecular Genetics and Metabolism. 2001; 74(1–2):89–97. [PubMed: 11592806]
14. Raskind WH, Conrad EU, Chansky H, Matsushita M. Loss of heterozygosity in chondrosarcomas for markers linked to hereditary multiple exostoses loci on chromosomes 8 and 11. Am J Hum Genet. 1995; 56:1132–1139. [PubMed: 7726169]
15. Shojaee S, Sina F, Banihosseini SS, et al. Genome-wide linkage analysis of a Parkinsonian-pyramidal syndrome pedigree by 500 K SNP arrays. Am J Hum Genet. 2008; 82(6):1375–1384. [PubMed: 18513678]
16. Ott J. Computer-simulation methods in human linkage analysis. Proc Natl Acad Sci U S A. 1989; 86(11):4175–4178. [PubMed: 2726769]
17. Weeks DE, Ott J, Lathrop GM. SLINK: a general simulation program for linkage analysis. A J Med Genet. 1990; 47:A204.
18. Schaffer AA, Gupta SK, Shriram K, Cottingham RW Jr. Avoiding recomputation in linkage analysis. Hum Hered. 1994; 44(4):225–237. [PubMed: 8056435]
19. Strauch K, Fimmers R, Kurz T, Deichmann KA, Wienker TF, Baur MP. Parametric and nonparametric multipoint linkage analysis with imprinting and two-locus-trait models: application to mite sensitization. Am J Hum Genet. 2000; 66(6):1945–1957. [PubMed: 10796874]
20. Broman KW, Murray JC, Sheffield VC, White RL, Weber JL. Comprehensive human genetic maps: individual and sex-specific variation in recombination. Am J Hum Genet. 1998; 63:861–869. [PubMed: 9718341]
21. Poorkaj P, Nutt JG, James D, et al. parkin mutation analysis in clinic patients with early-onset Parkinson's disease. Am J Med Genet A. 2004; 129A(1):44–50. [PubMed: 15266615]
22. Zabetian CP, Yamamoto M, Lopez AN, et al. LRRK2 mutations and risk variants in Japanese patients with Parkinson's disease. Mov Disord. 2009; 24(7):1034–1041. [PubMed: 19343804]
23. Nolte D, Niemann S, Muller U. Specific sequence changes in multiple transcript system DYT3 are associated with X-linked dystonia parkinsonism. Proc Natl Acad Sci U S A. 2003; 100(18):10347–10352. [PubMed: 12928496]

24. Braak H, Braak E. Neuropathological staging of Alzheimer-related changes. Acta Neuropathol. 1991; 82:239–259. [PubMed: 1759558]
25. Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. Neurobiol Aging. 1997; 18(4 Suppl):S1–2. [PubMed: 9330978]
26. Markopoulou K, Dickson DW, McComb RD, et al. Clinical, neuropathological and genotypic variability in SNCA A53T familial Parkinson's disease: Variability in familial Parkinson's disease. Acta Neuropathol. 2008; 116(1):25–35. [PubMed: 18389263]
27. Makino S, Kaji R, Ando S, et al. Reduced neuron-specific expression of the TAF1 gene is associated with X-linked dystonia-parkinsonism. Am J Hum Genet. 2007; 80(3):393–406. [PubMed: 17273961]
28. Pankratz N, Nichols WC, Uniacke SK, et al. Genome screen to identify susceptibility genes for Parkinson disease in a sample without parkin mutations. Am J Hum Genet. 2002; 71(1):124–135. [PubMed: 12058349]
29. Dickson DW, Bergeron C, Chin SS, et al. Office of rare diseases neuropathologic criteria for corticobasal degeneration. Journal of Neuropathology and Experimental Neurology. 2002; 61(11): 935–946. [PubMed: 12430710]
30. Houlden H, Baker M, Morris HR, et al. Corticobasal degeneration and progressive supranuclear palsy share a common tau haplotype. Neurology. 2001; 56(12):1702–1706. [PubMed: 11425937]
31. Ramirez A, Heimbach A, Grundemann J, et al. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nat Genet. 2006; 38(10): 1184–1191. [PubMed: 16964263]
32. Di Fonzo A, Dekker MC, Montagna P, et al. FBXO7 mutations cause autosomal recessive, early-onset parkinsonian-pyramidal syndrome. Neurology. 2009; 72(3):240–245. [PubMed: 19038853]
33. Davison C. Pallido-pyramidal disease. J Neuropathol Exp Neurol. 1954; 13(1):50–59. [PubMed: 13118374]
34. Mellick GD, Buchanan DD, McCann SJ, et al. Variations in the monoamine oxidase B (MAOB) gene are associated with Parkinson's disease. Mov Disord. 1999; 14(2):219–224. [PubMed: 10091612]
35. Hebert SS, De Strooper B. Molecular biology. miRNAs in neurodegeneration. Science. 2007; 317(5842):1179–1180. [PubMed: 17761871]

Figure 1. The four-generation pedigree of the XPDS family

A diagonal line denotes individuals who are deceased. Haplotypes are shown for all pedigree members from whom DNA was obtained. The apparent disease-related haplotype region is boxed. Haplotype markers are listed to the right of the genotype for subject II-9 and approximate distances between markers are listed to the right. Secondary genotyping defined the critical region between markers DXS8042 and DXS1216; DX1216 is located between markers DXS991 and DXS986.

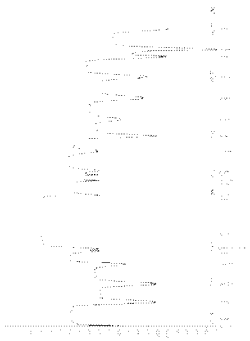

Figure 2.
Multipoint linkage analysis performed with GENEHUNTER-IMPRINTING using 23 markers spanning the X chromosome.

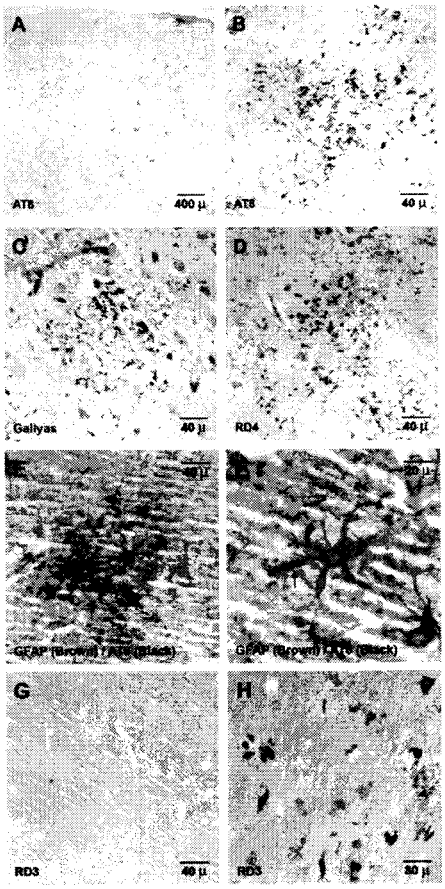

Figure 3. Immunostaining characteristics of plaque-like pathology in the striatum of the XPDS autopsied case

AT8, Gallyas, and RD4 (4-repeat tau specific antibody) stain plaque-like pathology in the striatum (A–D). Double immunolabeling with GFAP for astrocytes and AT8 demonstrate close association of glia and tau pathology (E) and double labeling of glial processes (F, arrow). Immunostaining with the 3-repeat tau specific antibody RD3 fails to label this pathology in the striatum (G), although age-associated neurofibrillary tangle and neuritic plaque pathology in the hippocampus was immunopositive (H).

Table 1

Clinical characteristics of the affected XPDS subjects.

Pedigree No.	II-2	II-9	III-8	IV-1	IV-5
Gender	M	M	M	M	M
Age at onset, years Mean, 39.2 ± 16.8 Range, 14 –58	54	58	44	26	14
Age: death (D) or current, years; Mean, 59.8 ± 20.3 Range, 36–86	D 86	78	61	36	38
Cogwheel rigidity	+	+	+	+	+
Resting tremor	+	+	+	+	+
Bradykinesia	+	+	+	–	+
Masked facies	+	+	–	+	+
Hyperactive tendon response	–	–	+	+	+
Babinski sign	?	–	+	+	+/–
Medication	l-dopa: 2,000 mg/day	seligiline: 5 mg/day	l-dopa	-	l-dopa
Miscellaneous	Columbia parkinsonism score=22 (70 yo); UPDRS score=16 (76 yo); Moderate memory loss late in disease.	UPDRS score=18 (66 yo); Brain CT age 58: normal.	–	normal MRI (brain/spinal cord)	+ Fluoro-dopa PET; normal brain MRI; seizure disorder; nystagmus; deep brain stimulation.

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Table 2

Candidate genes screened in the XPDS critical region.

Gene	Accession #	start-end coordinates	Gene function/DISEASE
GPR34	NM_005300	41433169-41441474	G-protein coupled receptor, highly expressed in brain, up-regulated in activated microglia
FUNDC1	NM_173794	44267848-44287160	Expressed in brain, function unknown
CHST7	NM_019886	46318135-46342781	Sulfotransferase, low levels of expression in brain
NDUFB11	NM_019056	46886562-46889381	NADH dehydrogenase (ubiquinone) 1 beta
PCTK1	NM_033018	46962575-46974336	Protein kinase, highly expressed in brain
TIMM17B*	NM_005834	48635675-48640370	Translocase of inner mitochondrial membrane
KCND1	NM_004979	48703582-48743719	Potassium voltage-gated channel, highly expressed in brain
GRIPAP1*	NM_020137	48715077-48743619	Highly expressed in nervous system, may regulate AMPA receptor distribution
SYP	NM_003179	48931208-48943605	Neuronal integral membrane protein in small synaptic vesicles
TMEM29	NM_014138	52944586-52954308	Transmembrane protein 29, highest expression in brain
GPR173	NM_018969	53095230-53123967	G-protein coupled receptor, highly expressed in brain, super-conserved
UBQLN2	NM_013444	56606797-56610102	N-terminal ubiquitin-like domain C-terminal ubiquitin-associated domain
CASK	**NM_003688**	**41264287-41667212**	**MENTAL RETARDATION AND MICROCEPHALY**
UBA1*	**NM_153280**	**46935142-46959471**	**SPINAL MUSCULAR ATROPHY, INFANTILE X-LINKED**
PORCN	**NM_203476**	**48252314-48264146**	**FOCAL DERMAL HYPOPLASIA**
SYN1*	**NM_006950**	**47316244-47364200**	**RETT SYNDROME**
EFNB1	**NM_004429**	**67965555-67982754**	**CRANIOFRONTONASAL SYNDROME**

*Coding sequence single nucleotide polymorphisms identified.

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Altered splicing of *ATP6AP2* causes X-linked parkinsonism with spasticity (XPDS)

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We report a novel gene for a parkinsonian disorder. X-linked parkinsonism with spasticity (XPDS) presents either as typical adult onset Parkinson's disease or earlier onset spasticity followed by parkinsonism. We previously mapped the XPDS gene to a 28 Mb region on Xp11.2–X13.3. Exome sequencing of one affected individual identified five rare variants in this region, of which none was missense, nonsense or frame shift. Using patient-derived cells, we tested the effect of these variants on expression/splicing of the relevant genes. A synonymous variant in *ATP6AP2*, c.345C>T (p.S115S), markedly increased exon 4 skipping, resulting in the overexpression of a minor splice isoform that produces a protein with internal deletion of 32 amino acids in up to 50% of the total pool, with concomitant reduction of isoforms containing exon 4. ATP6AP2 is an essential accessory component of the vacuolar ATPase required for lysosomal degradative functions and autophagy, a pathway frequently affected in Parkinson's disease. Reduction of the full-size ATP6AP2 transcript in XPDS cells and decreased level of ATP6AP2 protein in XPDS brain may compromise V-ATPase function, as seen with siRNA knockdown in HEK293 cells, and may ultimately be responsible for the pathology. Another synonymous mutation in the same exon, c.321C>T (p.D107D), has a similar molecular defect of exon inclusion and causes X-linked mental retardation Hedera type (MRXSH). Mutations in XPDS and MRXSH alter binding sites for different splicing factors, which may explain the marked differences in age of onset and manifestations.

INTRODUCTION

Recently, we described a family (Fig. 1) affected with a distinct X-linked parkinsonian syndrome, XPDS (1). XPDS is a slowly progressive disease with considerable phenotypic variability with respect to age of onset (range: 14–58 years) and presenting symptoms. Spasticity was the initial symptom in three affected individuals who later developed a parkinsonian resting tremor, masked facies and bradykinesia. Two individuals presented with typical parkinsonian features and did not manifest spasticity, even at old age. One affected individual developed seizures and one had moderate memory loss in his 80s, but none had developmental delay. We mapped the XPDS locus to a 28 Mb region on chromosome Xp11.2–Xq13.3 that contains ~200 protein coding genes (1). Complete or partial sequencing of 18 selected candidate genes failed to reveal a pathological change.

Analysis of genetic variation in families via exome resequencing has proved a powerful approach for identifying genes that

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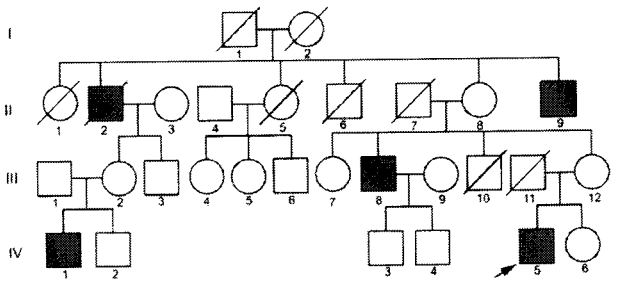

Figure 1. Family with X-linked parkinsonism and spasticity (XPDS). The individual tested via exome sequencing is designated with an arrow.

underlie Mendelian disorders. It has been successful even in challenging circumstances, including rare disorders with a limited number of subjects, presence of locus heterogeneity and/or reduced penetrance (2,3). These studies have relied on an exceedingly rare frequency of the causative variant in the population. Here we report functional analysis of candidate variants detected by exome resequencing that identified the genetic cause of XPDS.

RESULTS

Identification and functional analysis of candidate variants

Exome sequencing revealed 100 variants (91 SNPs, 9 indels) in the identity-by-descent (IBD) region on the X chromosome. Assuming that XPDS is an extremely rare disorder, we filtered out variants annotated in dbSNP or the 1000 Genomes Database as of June 2011, or were present in 1200 Caucasian exomes (NHLBL Exome Variant Server; UW EVS). This reduced the number of candidate variants to five, of which one was synonymous, two resided in a 3′-UTR and two were intronic indels within 30 nt from an exon–intron boundary (Table 1). Sanger sequencing was performed to validate the exome data and confirm co-transmission with disease.

In parallel, given the pattern of decreasing age at onset and increasing severity of symptoms with successive generations in the XPDS family, we tested possible repeat expansions in the coding sequence within the linkage region. Repeat instability within non-coding regulatory sequences tends to produce very variable phenotypes, whereas repeat expansions within coding regions more typically produce a core phenotype with increased severity (4) as is manifested in this family. No expansions were detected in the re-aligned exome reads from the IBD interval. The reference genome contained ~3700 potentially unstable repeats (total length ≥24 nt) within genic regions of the IBD interval, of which 10 resided in coding exons and/or exon–intron boundaries. All 10 sites were verified by Sanger sequencing in two affected subjects (IV-1 and IV-5) and two control subjects, but no repeat expansions were found (data not shown).

Given the absence of mutations that directly affect protein sequence, we next evaluated the effect of the five variants on gene expression and RNA splicing using patient-derived lymphoblastoid cell lines (LCL). For an X-linked disorder, even modest changes in gene expression/RNA splicing may be sufficient to cause disease in males who lack a normal allele. By qRT–PCR, we tested the two variants in 3′ UTRs for changes in the gene-expression level. We also examined an intronic variant near the exon–intron boundary and a synonymous exonic variant for evidence of altered splicing, such as changes in the balance of splice isoforms or appearance of new products.

Using LCLs from two patients and two unaffected Caucasian males, we did not find consistent effects of variants in *RGN* and *XAGE3* on mRNA expression level or the variant in *MED14/EXLM1* on splicing. *PAGE5* was not expressed in LCL. In contrast, the silent exonic c.345C>T (p.S115S) mutation dramatically altered splicing of the *ATP6AP2* gene (MIM 300556) (Fig. 2).

In addition to a major splice product of the expected size that migrates at 250 bp, a faint minor band at 150 bp is seen in both controls. This 150 bp band becomes a major species in both XPDS subjects. Direct sequencing of the eluted and purified RT–PCR fragments determined that the 250 bp band contains normally spliced exons 3, 4 and 5, whereas the 150 bp band lacks exon 4. The skipping of exon 4 results in an in-frame transcript (Δe4) encoding a protein with internal deletion of 32 residues. The upper band seen in both patients consists of a heterogeneous mixture of transcripts and is likely an RT–PCR artifact.

ATP6AP2 is an essential gene with ubiquitous expression. It encodes a single-pass transmembrane domain protein that is involved in a range of processes such as intracellular pH homeostasis (5), renin–angiotensin system (6) and WNT signaling (7). Surprisingly, another mutation in this gene causes the MRXSH syndrome (OMIM #300423), a congenital mental retardation with epilepsy (8). This silent mutation, c.321C>T (p.D107D), also positioned in exon 4, significantly impairs splicing efficiency resulting in the overexpression of the Δe4 transcript.

Variants in *ATP6AP2* exon 4 and their predicted effect on splicing

The nucleotide sequence of exon 4 is nearly invariant in the human population. Besides mutations found in the MRXSH and XPDS families, there is only one rare synonymous c.357G>A (p.E119E) variant (0.02% frequency) listed in the EVS. No phenotypic information was available for this sample. We found no exon 4 mutations in 1160 patients with Parkinson's disease (PD). However, only 35 male patients had a family history consistent with an X-linked disorder (e.g. two or more affected males, no male-to-male transmission) and none had a history of spasticity.

Human Splicing Finder predictions suggest that the two disease-related mutations, c.321C>T (p.D107D) and c.345C>T (p.S115S), affect different sets of splicing factors (Table 2). c.321C>T (p.D107D) disrupts enhancer sites for SRp40 and 9G8, whereas c.345C>T (p.S115S) creates a new silencer site. Interestingly, c.357G>A (p.E119E) could also affect splicing of exon 4, although through different mechanisms. c.357G>A (p.E119E) is predicted to disrupt both a potential enhancer for splicing factor SRp55 and a silencer for hnRNP A1.

Table 1. Unique variants found in the XPDS exome within the X-chomosome linkage interval

Variant (hg19 coordinates)	Description	Gene symbol	Gene name
40 456 545 C>T	Coding, synonymous	ATP6AP2	ATPase, H+ transporting, lysosomal accessory protein 2
55 250 449 A>G	3′-UTR, non-coding	PAGE5	P antigen family, member 5 (prostate associated)
46 952 353 G>A	3′-UTR, non-coding	RGN	regucalcin (senescence marker protein-30)
40 588 605 G>GA	Intronic indel	MED14, EXLM1	mediator complex subunit 14, transcription activator
52 891 619GT>G	Intronic indel	XAGE3	X antigen family, member 3

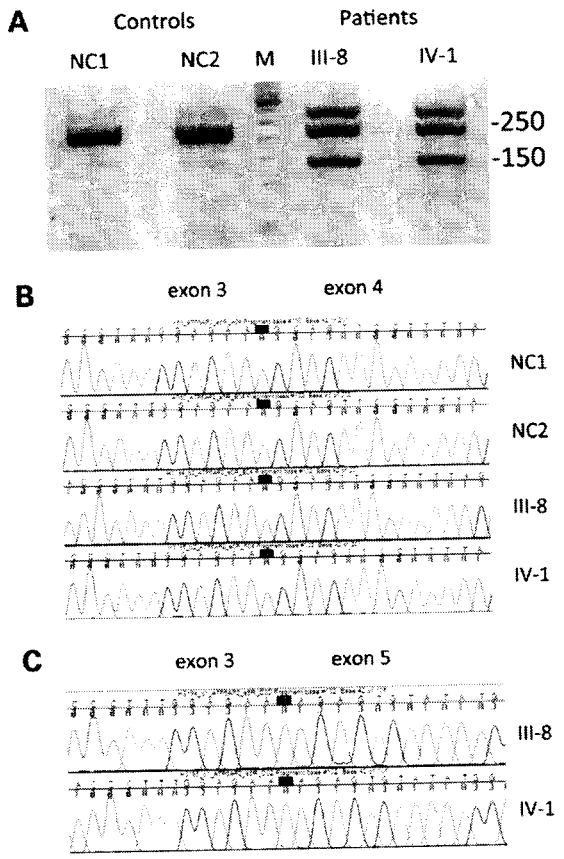

Figure 2. The silent c.345C>T (p.S115S) mutation results in overexpression of *ATP6AP2* splice isoforms that lack exon 4. (**A**) RT–PCR with primers positioned in exons 3 and 5 amplifies a major 250 bp and a minor 150 bp product in controls; the minor product is highly overexpressed in patients. (**B** and **C**) Sequence analysis of splicing products from 250 bp (B) and 150 bp bands (C) confirming exon 4 skipping in the 150 bp band. III-8, IV-1: affected patients from the XPDS pedigree depicted in Fig. 1. NC1, NC2: normal controls. M: molecular weight marker.

Overproduction of minor Δe4 isoform in XPDS cells compromises the level of normal full size transcript

According to the AceView database (http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/), the human *ATP6AP2* gene is alternatively spliced in multiple tissues including brain (Fig. 3A), and human *ATP6AP2* mRNA splice isoforms are much more complex than those of mice (Fig. 3B). There are two major *ATP6AP2* isoforms containing normally spliced exon 4 (a and d supported by 214 and 117 tissue-averaged cDNA clones, respectively, Fig. 3A), as well as several minor forms, including Δe4 (isoform c, 12 supporting clones). We used qRT–PCR quantification to measure the effect of the c.345C>T (p.S115S) variant on the relative proportion of *ATP6AP2* splice isoforms, as well as on the overall transcript level (Fig. 4). In normal individuals, blood-derived cells contained <1% of Δe4 transcript (average 0.4%, range 0.07–0.8%) while brain tissues produced it at a 10-fold higher level (average 4%, range 1.5–8.4%, Fig. 4A). Strikingly, the Δe4 level is increased over 90-fold in XPDS patients, becoming a major isoform in blood cells (average 44%; patients' averages of 35% in LCL and 50% in uncultured white cells). The observed increase in Δe4 production caused by c.345C>T (p.S115S) is comparable to that of the c.321C>T (p.D107D) mutation found in MRXSH (50% in LCL) (8).

We were interested in whether Δe4 overexpression increases total production of *ATP6AP2* mRNA or competes with the production of the isoforms that contain exon 4, resulting in their relative depletion. To account for total *ATP6AP2* mRNA production, we performed an additional quantification using exons 8 and 9 present in splice isoforms a through f (Fig. 3A). The expression of e8–e9, e3–e4 and Δe4 was compared with that of external reference genes (*GUSB* and *TBP*, Fig. 4B–D). We observed no differences in the amount of total *ATP6AP2* transcript between patients and controls (Fig. 4C). In XPDS patients, production of exon 4-containing isoforms was decreased, indicating that they indeed compete with Δe4 for pre-mRNA (Fig. 4B and D).

Deficit of ATP6AP2 protein in XPDS brain

To study the expression and distribution of ATP6AP2 protein in XPDS brain, we took advantage of available brain sections from the previously characterized patient II-2 (1). Sections from this brain and two age-matched control brains were stained with polyclonal antibodies raised against the extracellular domain of the ATP6AP2 protein (Fig. 5). In the normal brain, the cytoplasm and plasma membrane of the majority of neurons were stained positively. In the XPDS case, we observed a similar distribution of immunostaining, but with marked decrease in staining intensity in both the frontal cortex and the striatum; the difference was less pronounced in the hippocampus.

On the role of ATP6AP2 in Wnt/β-catenin signaling

ATP6AP2 has been shown to modify Wnt/β-catenin signaling and may act as an adaptor between Wnt receptors, Frizzled and Lrp5/6, and the V-ATPase complex (7,9). Because deregulated Wnt signaling is a frequent finding in neurological disorders (10), we tested whether modulation of ATP6AP2

expression and/or overexpression of the Δe4 isoform could influence Wnt/β-catenin signaling. We first depleted ATP6AP2 protein in HEK293T cells harboring a Wnt/β-catenin-activated luciferase reporter (BAR) (11). Of six siRNAs shown by western blot to reduce ATP6AP2 protein, three reduced the ability of Wnt3A conditioned media to activate Wnt signaling, one further enhanced Wnt3A-dependent signaling, and two had no effect (Supplementary Material, Fig. S1).

Overexpression of ATP6AP2 is thought to repress Wnt/β-catenin signaling (9); whereas, expression of ATP6AP2 lacking its C-terminus (ΔC) has been shown to synergize with Wnt3A to activate Wnt/β-catenin signaling (7). Thus, we tested whether overexpression of the Δe4 isoform of ATP6AP2 could also influence Wnt/β-catenin activation. Unlike positive control proteins, β-catenin and constitutively active LRP6 (12), we did not observe changes in BAR activity when full length ATP6AP2, ATP6AP2 ΔC or ATP6AP2 Δe4 were overexpressed (Supplementary Material, Fig. S2). Collectively, our findings suggest that ATP6AP2 does not significantly influence Wnt/β-catenin signaling.

Table 2. Predicted effect of variants in exon 4 of *ATP6AP2* on splicing

Phenotype	Variant	Exon Enhancer Site	Exon silencer site
XPDS	c.345C>T	−10.01% for SRp40	new site
MRXSH	c.321C>T	site broken for SRp40 site broken for 9G8	+22% for hnRNP A1
Unknown	c.357G>A	site broken for SRp55	site broken for hnRNP A1

ATP6AP2 deficiency affects V-ATPase function with resultant impaired autophagy and lysosomal clearance

ATP6AP2 is an essential accessory unit of the V-ATPase multiprotein complex responsible for a number of processes in the eukaryotic cell including endosome acidification, endocytosis and vesicular trafficking. The engineered ablation of ATP6AP2 in cardiomyocytes leads to disassembly of the V-ATPase complex, loss of its function, impaired autophagy and eventually cell death, (13) mimicking the effect of V-ATPase inhibitors, such as bafilomycin A1 (BafA1). We were interested whether partial depletion of this essential protein by siRNA could

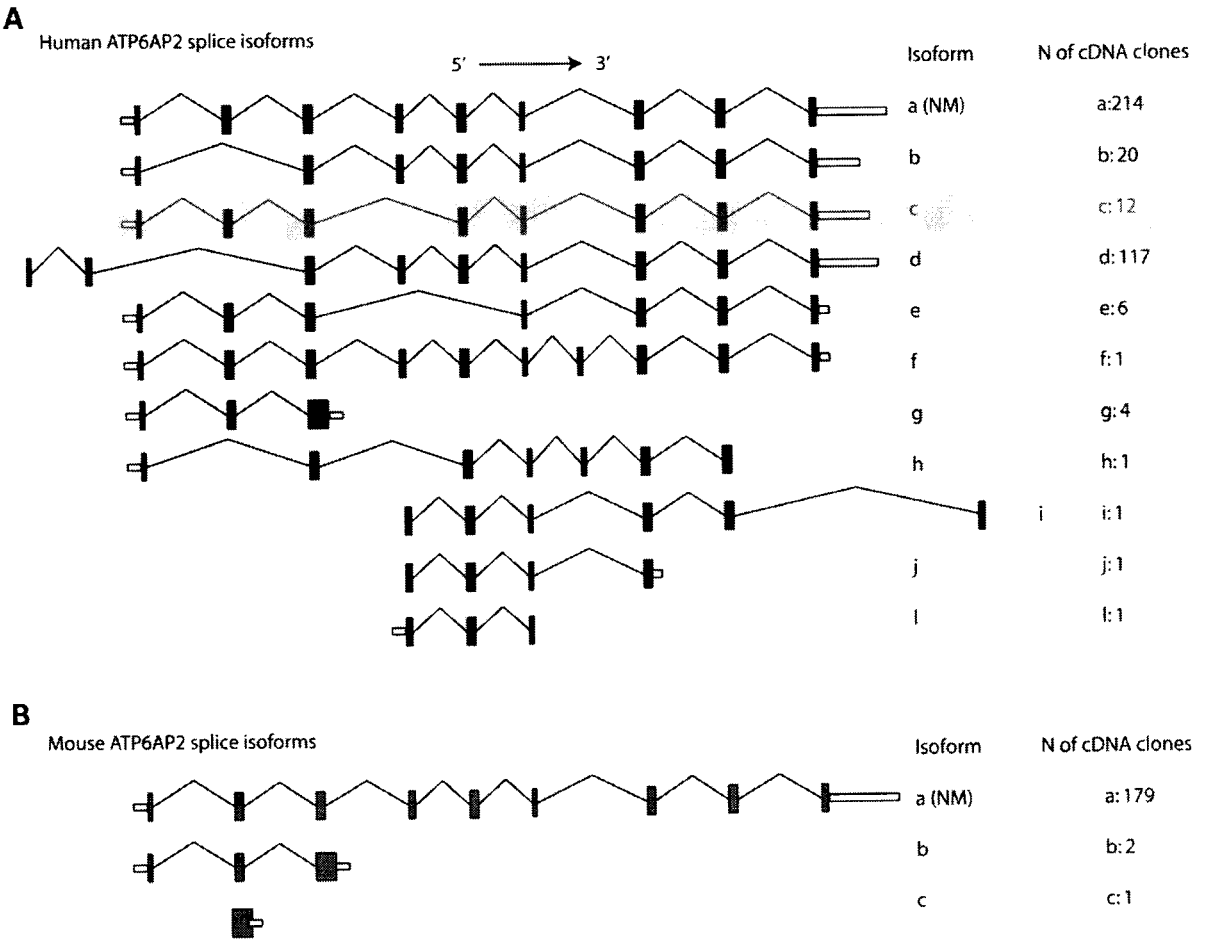

Figure 3. Distribution of *ATP6AP2* splice isoforms in humans (**A**) and mice (**B**). The shaded box highlights the minor isoform c that corresponds to the Δe4 isoform increased in XPDS patients. (NM), isoform corresponding to RefSeq gene: NM_005765 (human) and NM_027439 (mouse).

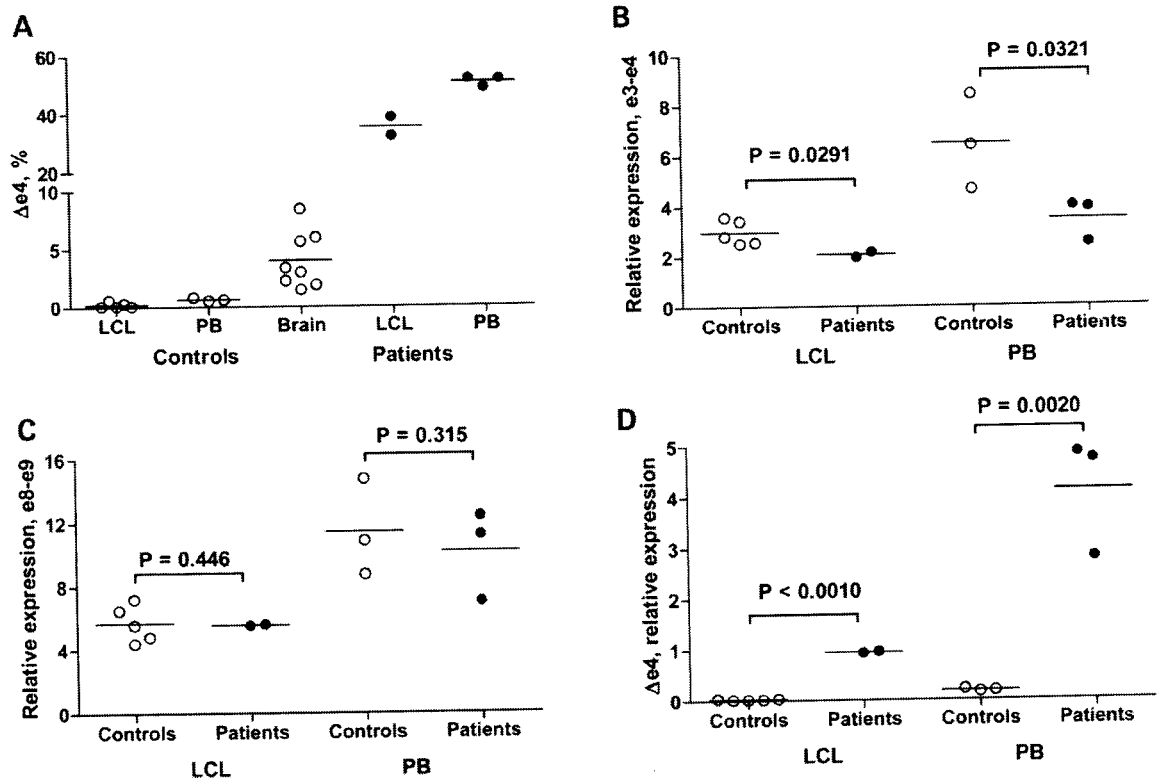

Figure 4. qRT–PCR quantification of *ATP6AP2* splice isoforms in XPDS patients and controls. (**A**) Percentage of the Δe4 *ATP6AP2* isoform in blood cells and brain tissues. (**B**) Relative expression of *ATP6AP2* isoforms containing exon 4. (**C**) Relative expression of total *ATP6AP2* transcript as measured with primers for constant exons 8 and 9. (**D**) Relative expression of Δe4 isoform. Open circles, controls; filled circles, patients. PB, uncultured peripheral blood cells.

produce a V-ATPase-deficiency related phenotype. High concentration of BafA1 (>100 nM) inhibits V-ATPase completely, induces vacuolar deacidification that impairs lysosomal protein degradation (14) and promotes apoptosis (15). We therefore investigated whether ATP6AP2 knockdown by siRNA would synergize with BafA1 at concentrations below its reported ability to inhibit vesicular acidification. All three siRNAs to ATP6AP2 shown to efficiently deplete the protein (Supplementary Material, Fig. S1) decreased cell survival at low doses of BafA1 (Fig. 6A). Next, we examined the effect of siRNA knockdown on autophagy using the expression of LC3 and p62 as the read-outs. Conversion of LC3-I (cytosolic form) to LC3-II (membrane-bound lipidated form) is a measure of autophagosomal formation. We evaluated the LC3-II/LC3-I ratio under conditions that either induce or block autophagy (Fig. 6B). In cells where autophagy was induced by starvation or blocked at the fusion step by BafA1 the ratio was increased at a comparable level; the combined treatment (starvation followed by BafA1) augmented the LC3-II/LC3-I ratio in an additive manner. We did not observe significant differences in autophagic flux between control and ATP6AP2 knockdown cells.

Another common method of autophagy evaluation is visualization of autophagosomes as LC3-positive puncta. We used HEK293 cells stably transfected with the mRFP-GFP-LC3 reporter (16). The difference in pH sensitivity of the two fluorescent tags allows the autophagosome before fusion with the lysosome (yellow fluorescence of both RFP and GFP) to be distinguished from the autolysosome (red fluorescence of RFP). All three siRNAs to ATP6AP2 augmented the presence of punctate LC3-positive structures (Fig. 6C). Accumulation of yellow and red LC3-positive puncta indicates perturbation of autophagy and lysosomal clearance.

To examine the XPDS brain for defects in autophagy, we performed comparative immunostaining with p62/SQSTM1 antibodies, a marker of impaired autophagy in various neurodegenerative conditions (17). IHC revealed massive accumulation of p62 in the XPDS striatum (Fig. 7) but not in other regions examined (data not shown) indicating a region-specific impairment of constitutive autophagy in the XPDS brain. It is of note that ATP6AP2-deficiency was also most prominent in the XPDS striatum (Fig. 5).

DISCUSSION

Herein, we show that a silent mutation in *ATP6AP2*, which encodes an accessory unit of an essential lysosomal enzyme, V-ATPase, is the cause of a familial parkinsonian disorder, XPDS. The availability of patient blood cells allowed detection of the underlying molecular phenotype, aberrant splicing of *ATP6AP2* mRNA that results in the overexpression of its minor isoform. Another silent mutation in the same exon of

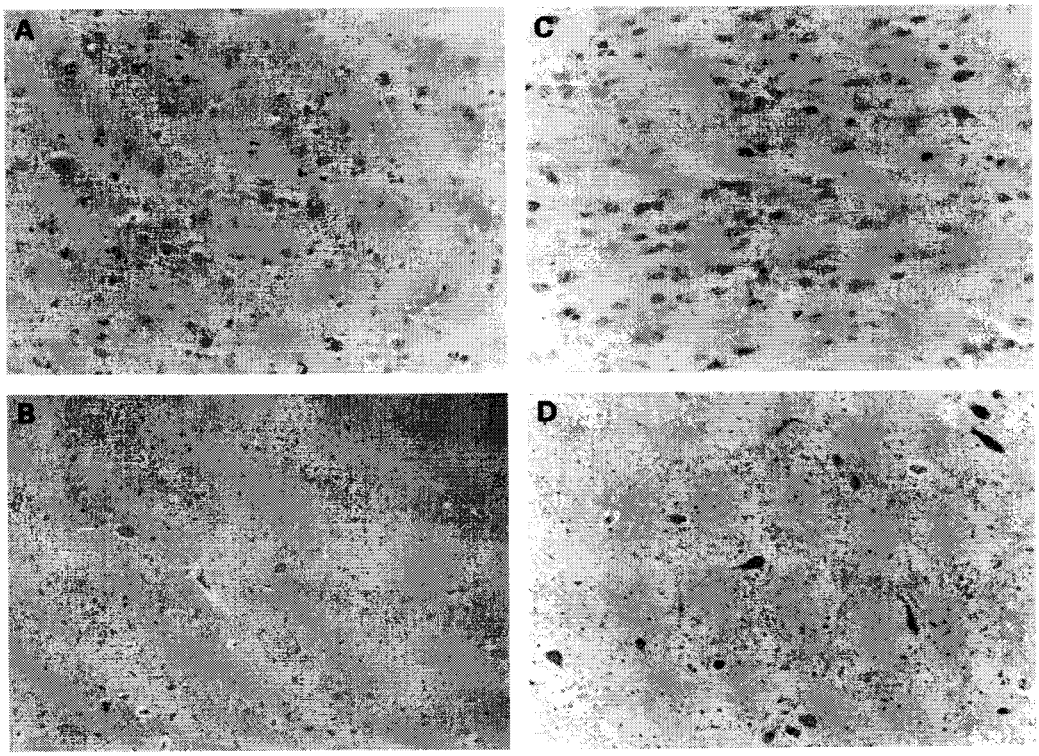

Figure 5. Reduced ATP6AP2 immunostaining in the XPDS brain. Representative immunostaining of brain sections of the XPDS case (**A** and **B**) and controls (**C** and **D**). In both the frontal cortex (A and C) and striatum (B and D), the XPDS case demonstrated normal distribution but marked reduction in the intensity of ATP6AP2 immunostaining in neurons.

ATP6AP2 that produces a similar defect in splicing causes X-linked mental retardation Hedera type (MRXSH; OMIM 300423), a distinct CNS pathology with several overlapping features with XPDS, which include spasticity and seizures (1,8,18). According to recent estimates, at least 15% of all disease-causing mutations affect RNA splicing (19,20). Many of them are silent exonic mutations with no clearly predictable effect on splicing. This highlights a necessity to procure cells or other biological material from patients for studies of genetic disorders.

V-ATPase complex and its accessory protein ATP6AP2 are present within the plasma membrane and membranes of some organelles such as endosome, lysosome and secretory vesicle. V-ATPase is involved in receptor-mediated endocytosis, membrane trafficking, protein processing and degradation and nutrients uptake (21,22). V-ATPase complex is composed of an 8-subunit V1 sector that is responsible for ATP hydrolysis and a 6-subunit V0 sector that serves as a transmembrane proton channel (23,24). ATP6AP2 is specifically associated with the V0 sector ensuring its integrity. Targeted disruption of *ATP6AP2* is lethal to the cell; it destabilizes V0 and prevents its assembly but has no effect on V1 (13). The resulting acute V-ATPase deficiency carries multiple consequences including impaired lysosome-mediated protein degradation and autophagy (13,25). We show that even partial depletion of ATP6AP2 by siRNA sensitizes HEK293T cells to low doses of V-ATPase pump inhibitor BafA1. The accumulation of autophagosomes and autolysosomes upon siRNA knockdown suggests a direct effect of ATP6AP2 deficiency on the autophagy process. The immunoblotting with endogenous LC3 did not detect autophagy changes; the results from the two LC3-based assays may reflect differences in assay sensitivity and/or cell-line-specific responses and warrant further investigation. Perhaps most relevant to the pathogenesis of the disorder, the most affected area of XPDS brain, the striatum, showed ATP6AP2 deficiency, pathological Tau deposits (1) and massive accumulation of p62/SQSTM1 indicating profound defect in lysosome-mediated protein degradation and autophagy.

ATP6AP2 is a ubiquitous protein as is its pathological Δe4 ATP6AP2 isoform, which is overexpressed in XPDS and MRXSH cells of non-neural origin. Yet, the effect of mutations in both disorders is CNS-confined rather than pleiotropic. This may be explained by particularly strong demand of neurons for V-ATPase related functions. Additionally, ATP6AP2 isoforms may carry out yet unidentified functions exclusive for neural cells. The complexity of alternative splicing of *ATP6AP2* has greatly evolved in the human compared with the mouse (Fig. 3). In the PC-12 model of neurogenesis, overexpression of Δe4 ATP6AP2 inhibited neural differentiation (26). Besides overexpression of Δe4, a splicing mutation ultimately affects the level of the normal full size *ATP6AP2* isoform. At the transcript level, the production of the normal isoform is reduced because of competition for pre-mRNA. At the protein level, the Δe4 isoform seems to have a shorter half-life (8,26) resulting in a lowered output of total ATP6AP2 protein. In normal brain, basal Δe4 expression is an order of magnitude higher than in non-

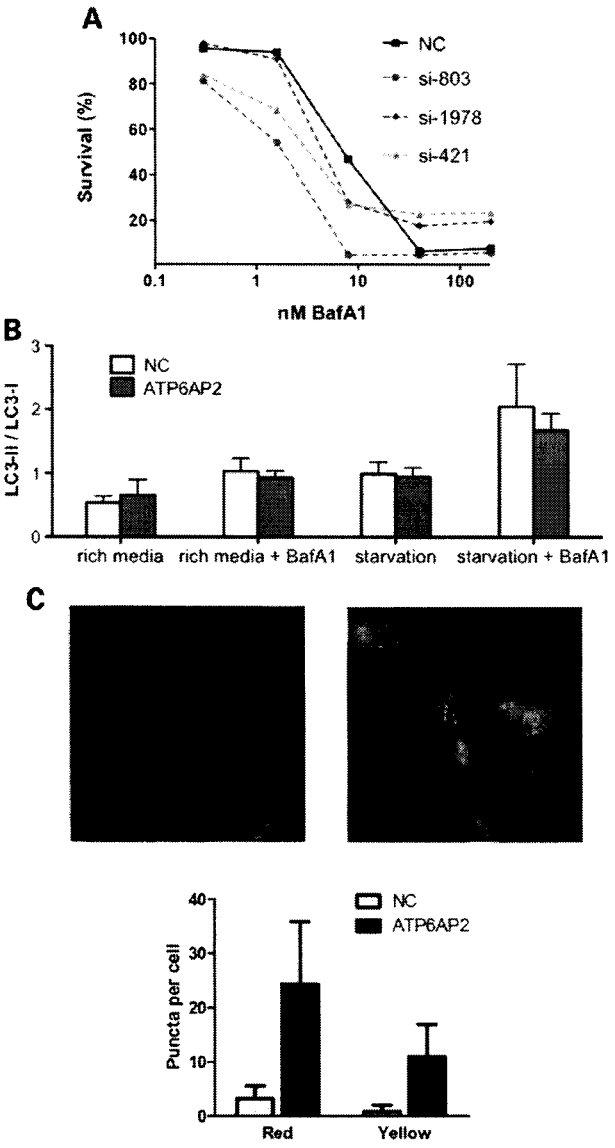

Figure 6. Knockdown of ATP6AP2 affects V-ATPase function and sensitizes cells to low concentrations of BafA1 (**A**). Dose–response of HEK293T cells to BafA1 after ATP6AP2 knockdown by siRNA. Forty-eight hours after transfection with three different siRNAs targeting ATP6AP2 (si-803, si-421, si-1978) or negative control siRNA (NC), HEK293T cells were exposed to BafA1 at the concentrations indicated. Cell-viability data are normalized to controls treated with 0.5% DMSO. (**B**) Autophagy flux measured in three independent experiments. HEK293T cells were transfected with negative control siRNA (NC) or with a pool of ATP6AP2 siRNA (ATP6AP2) and assayed for autophagy for a total of 6 h. BafA1 was added during the last 2 h of incubation. (**C**). ATP6AP2 deficiency induces accumulation of LC3-positive puncta. Shown is a representative ATP6AP2 siRNA knockdown experiment in HEK293 cells stably expressing the ptfLC3 reporter. Left panel, untransfected cells (NC); right panel, cells 96 h after transfection with a pool of ATP6AP2 siRNA (ATP6AP2); bottom, statistical analysis of puncta counts was performed on untreated ($N = 31$) and ATP6AP2 siRNA treated cells ($N = 21$) using a two-tailed t-test ($P < 0.0005$) for both autolysosomes (red puncta) and autophagosomes (yellow puncta).

neural cells so one can reasonably anticipate that the effect of the XPDS mutation on ATP6AP2 protein level in the brain will be also augmented. By IHC, we did observe a deficit of total ATP6AP2, which was most pronounced in the XPDS striatum, an area commonly involved in PD. Of note, heterozygous female carriers in both MRXSH and XPDS families do not manifest clinical symptoms even though the Δe4 transcript is overexpressed in their blood cells [(8) and our unpublished data]. The cells that express the normal *ATP6AP2* allele may be sufficient to compensate for cells with the mutant allele on the active X chromosome. It is also possible that disease-free female carriers have a skewed pattern of X inactivation in neural tissues.

Why do splicing mutations that produce a similar molecular phenotype in blood cells cause such different CNS disorders? The most likely explanation is a quantitative difference in Δe4 expression level and/or in the balance of ATP6AP2 splicing isoforms in the developing brain of MRXSH and XPDS patients. Although point mutations in the canonical 5′ and 3′ splice sites often have severe splicing phenotypes, exonic point mutations that cause efficient exon skipping are rare. This suggests that exon 4, by natural design, is balanced between inclusion and exclusion that always produces transcripts with a correct reading frame. The ratio of inclusion to exclusion would then depend on the developmental and tissue specific control of splicing factors. For many pathogenic splicing mutations, change in the isoform level is the tissue-specific quantitative trait. Examples are *MAPT*-related tauopathies (27), in which splicing mutations in *MAPT* result in rather subtle changes in the balance of splice isoforms of tau protein, and the pathology is confined to the CNS. The differential effect of *ATP6AP2* splicing mutations can be viewed as a result of interaction of corresponding *cis*-elements affected by the mutation with development stage-specific splicing factors in the brain. For instance, c.321C>T (p.D107D) disrupts a *cis*-element for several positive regulators and produces a permanent splicing defect in MRXSH patients. On the other hand, c.345C>T (p.S115S) creates a new silencer site. If the respective negative splicing regulator is temporarily downregulated in the developing brain, this would mitigate an early effect on neurodevelopment, but later might cause PD-like symptoms. It remains to be seen whether the affected individuals from the MRXSH family, who are currently very young, will develop PD-like features in adulthood.

MATERIALS AND METHODS

Subjects

Approval for the recruitment and genetic analysis of the XPDS family, control subjects and individuals with PD was granted by the University of Washington and VA Puget Sound Health Care System Institutional Review Boards. All tissue samples were obtained following written informed consent for autopsy and the use of the material and clinical information for research. The three living affected males and two obligate carrier females in the previously reported XPDS family were re-examined. Subjects with PD were participants in the Parkinson's Genetic Research Study (28) at the VA Puget Sound Health Care System. Unrelated participants from other studies, who provided

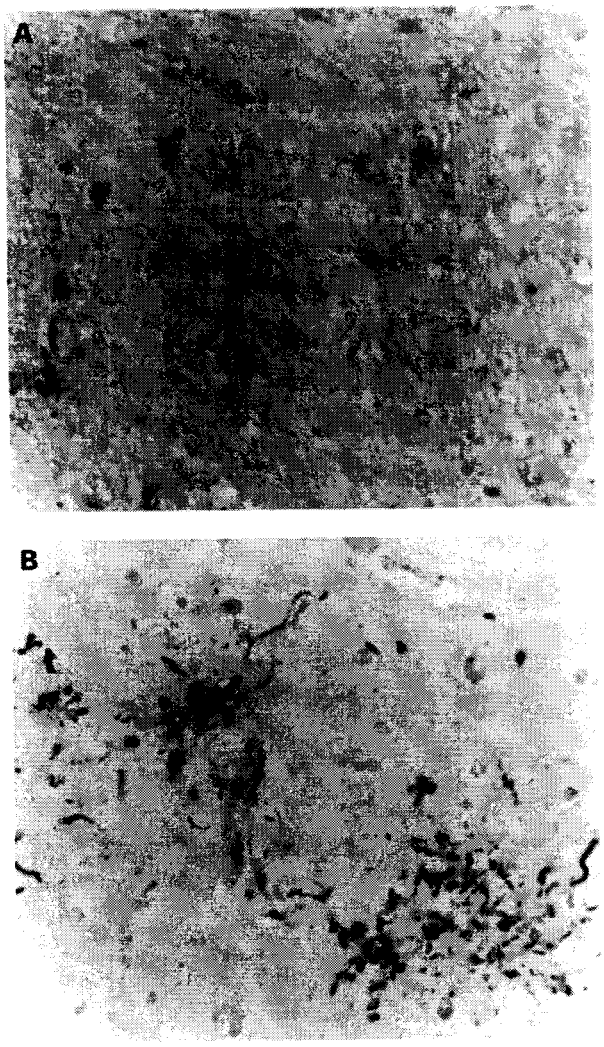

Figure 7. p62 immunostaining of the striatum of a control (**A**) and XPDS case (**B**). Note p62 labeling of plaque-like pathology, similar to tau immunostaining previously described in this case (1).

informed consent for sharing DNA or genetic information for use in research, served as controls. These individuals were not screened for PD.

Exome sequencing

Genomic DNA isolated from the blood of the most severely affected individual, IV-5 (1) (Fig. 1), was used to prepare a shotgun sequencing library. The Nimblegen_solution_V2refseq2010.HG19 probe library was used for target enrichment, and sequencing was performed on the Illumina GAIIx platform with paired-end 76 base reads. Fastq sequence files were aligned against the human reference sequence (National Center for Biotechnology Information 37/hg19) with the Burrows-Wheeler Aligner. Duplicate paired-end reads were removed from the merged data sets. SNP and indel calling was performed with the GATK Unified Genotyper and annotated with SeattleSeq server (http://gvs.gs.washington.edu/SeattleSeqAnnotation/) according to the National Center for Biotechnology and University of California Santa Cruz (http://genome.cse.ucsc.edu/cgi-bin/hgGateway) databases. Additionally, exome reads from the linkage interval on the X chromosome were re-aligned using the Splitread algorithm (29) to identify nucleotide repeat sequences within coding sequences and near intron/exon junctions. Tandem Repeats Finder (http://tandem.bu.edu/trf/trf.html) (30) and Short Tandem Repeat DNA Internet DataBase (https://tandem.bu.edu/cgi-bin/trdb/trdb.exe) (31) were used to screen the reference genome within the IBD interval for potentially unstable repeats, defined as having length 24 nt or more.

Cell lines and brain tissues

EBV-transformed LCL from patients and controls were established and maintained according to standard protocols (32). Human embryonic kidney cells (HEK-293 and HEK-293T) were obtained from the American Type Culture Collection and cultured in DMEM supplemented by 10% FBS at 37°C and 5% CO_2. Post-mortem brain tissues of neurologically normal control subjects used for RNA isolation were obtained from the Neuropathology Core Brain Bank at the University of Washington. The average age of subjects was 70 and the average post-mortem interval was 4 h. Tissue samples were flash frozen at the time of autopsy and stored at −80°C.

RNA isolation and cDNA synthesis

Total RNA was extracted from whole blood using Pure Link Total RNA Blood Kit (Invitrogen, Carlsbad, CA, USA), from cultured LCL or fresh-frozen human cerebella using RNeasy Mini Kit (Qiagen, Valencia, CA, USA). cDNA synthesis with poly-dT primer was performed using the SuperScript III RT–PCR kit (Invitrogen). Primers for detection of splice variants were designed using Primer3 and are available on request. Effect of variants on splicing was evaluated using Human Splicing Finder, http://www.umd.be/HSF/ (33).

Quantitative reverse transcription PCR

RNA was isolated from cultured cells at least two times per sample. Quantitative reverse transcription (qRT)–PCR was performed on a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using ABI gene-expression assays. Experiments were carried out in triplicate with two external control genes (GUSB (Hs00939627_m1, ABI) and TBP (Hs00427620_m1, ABI) for normalization of gene expression. To quantify the expression of ATP6AP2 isoforms, the following ABI qPCR assays were used: (i) Hs00997140_g1 to amplify e3–e4 junction; (ii) Hs00997145_m1 to amplify the invariant e8–e9 junction; (iii) a custom made ATP6AP2_ Δe4 assay to amplify e3–e5 junction lacking e4.

The following primers/probes were used for ATP6AP2_ Δe4 assay: Forward primer 5′-AGGGAGTGAACAAACTGGC TCTA-3′; Probe 5′-6FAM_CCCCAGGCAGTGTC_MGB-3′; Reverse primer 5′- taccatatacactctattctccaaagggta-3′.

Immunohistochemistry of brain sections

Post-mortem brain tissue from one XPDS case (pedigree position II-2) and two neurologically normal control subjects was obtained from the Neuropathology Core Brain Bank at the University of Washington. Formaldehyde-fixed paraffin-embedded sections from the frontal lobe, striatum and hippocampus were de-paraffinized and autoclaved at 15 psi, 121°C for 20 min either in citrate buffer, pH 6.0 (ATP6AP2) or in Tris/EDTA, pH 9.0 (p62/SQSTM1) for antigen retrieval. Immunodetection was performed with goat polyclonal antibodies against ATP6AP2 (AF5716, R&D Systems, Minneapolis, MN, USA), mouse monoclonal antibodies against p62/SQSTM1 (D-3, sc-28359, Santa-Cruz, Dallas, TX, USA) and secondary anti-mouse and anti-goat antibodies (Vector Laboratories, Burlingame, CA, USA). The specificity of the antigen detection was ascertained by omitting the primary antibody.

LC3 immunodetection by western blotting

Forty-eight hours after siRNA transfection, HEK293T cells were cultured in conventional or nutrient-deprived media (Eagle's balanced salt solution) for 6 h total time; where indicated, 400 nM BafA1 was added during the last 2 h of incubation. Cells were lysed in 2% SDS sample buffer followed by sonication twice for 5 s on ice to obtain whole-cell lysate. The lysates were heated at 100°C for 5 min and cleared by centrifugation at 20 000 rpm for 5 min at 4°C. Lysates were resolved fractionated on Novex® Tris–Glycine polyacrylamide gel (Life Technology). After transfer to PDVF membrane (Life Technology, CA, USA), immunoblotting with following primary antibodies: rabbit anti-LC3 (Thermo Scientific, IL, USA), rabbit anti-SQSTM1/p62 (Cell Signaling, MA, USA), rabbit anti-GAPDH (Sigma-Aldrich, St Louis, MO, USA) and with anti-rabbit HRP-conjugated secondary antibodies (Life Technology, CA, USA) were performed. The protein bands were visualized by enhanced chemiluminescence (Thermo Scientific, IL, USA) under densitometry (BioChemi digital imaging system, UVP, CA, USA) and quantified with ImageJ software (NIH). GAPDH expression level served as a loading control. The data were averaged from triplicate experiments and analyzed by *T*-test.

siRNA knockdowns

Reverse transfections of HEK-293T cells in 24-well plates were performed using Lipofectamine™ RNAiMAX reagent (Invitrogen) and siRNAs at 10 nM concentration. We used three siRNAs targeting various ATP6AP2 exons: NM_005765_stealth_1978; NM_005765_stealth_421; NM_005765_stealth_803; and Negative control No. 2 siRNA. Sequences are provided in the Supplementary Material and Methods.

Cell-viability assay

The luminescent CellTiter-Glo cell viability assay (Promega, Madison, WI, USA) measuring cellular ATP level was performed in duplicate according to the manufacturer's protocol. Briefly, 5×10^3 cells were seeded on wells in white clear bottom 96-well plates (Greiner Bio-One, Germany). The next day, a series of concentrations of BafA1 (Sigma-Aldrich, St Louis, MO, USA) or DMSO was added to the wells. After 48 h of culture, CellTiter-Glo reagent was added to the wells; luciferase luminescence was measured on a FluoStar Omega plate reader (BMG LABTECH, Germany).

Fluorescence microscopy

ptfLC3, the plasmid encoding the tandem fluorescent reporter was obtained from AddGene (Addgene plasmid 21074) (16). tfLC3 expressing stable cell lines were constructed by transfecting HEK293 with GenePorter 2 (Genlantis) using the manufacturer's protocol (34). Geneticin (400 μg/ml) and Zeocin (100 mg/ml) were used to maintain selections.

Cells were seeded onto poly-D-Lysine coated (Sigma-Aldrich, St Louis, MO, USA) 12 mm round glass cover slips in 24-well plates. Cells were fixed for imaging 96 h after siRNA transfection. Microscopy was performed on a Delta Vision microscope (Applied Precision, Inc.) using a 60x oil-immersion objective, a sCMOS camera, and 2 × 2 binning. Image analysis, volume rendering and isolation of red/green co-localization into a blue color channel were performed using softWorx 6.0 Beta software. Image segmentation, color channel separation and puncta counts were conducted in Adobe Photoshop CS5.

WEB RESOURCES

The URLs for data presented herein are as follows:

1000 Genomes Project, http://www.1000genomes.org/page.php
Database of Genomic Variants, http://projects.tcag.ca/variation/
dbSNP homepage, http://www.ncbi.nlm.nih.gov/SNP/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/
SeattleSeq Annotation, http://gvs.gs.washington.edu/SeattleSeqAnnotation/
University of California Santa Cruz Human Genome Browser, http://genome.cse.ucsc.edu/cgi-bin/hgGateway
Tandem Repeats Finder, http://tandem.bu.edu/trf/trf.html
Short Tandem Repeat DNAInternet DataBase, https://tandem.bu.edu/cgi-bin/trdb/
Human Splicing Finder, http://www.umd.be/HSF
AceView database, http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/
NHLBI Exome Sequencing Project Exome Variant Server, http://evs.gs.washington.edu/EVS/

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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REFERENCES

1. Poorkaj, P., Raskind, W.H., Leverenz, J.B., Matsushita, M., Zabetian, C.P., Samii, A., Kim, S., Gazi, N., Nutt, J.G., Wolff, J. *et al.* (2010) A novel X-linked four-repeat tauopathy with Parkinsonism and spasticity. *Mov. Disord.*, **25**, 1409–1417.
2. Ng, S.B., Bigham, A.W., Buckingham, K.J., Hannibal, M.C., McMillin, M.J., Gildersleeve, H.I., Beck, A.E., Tabor, H.K., Cooper, G.M., Mefford, H.C. *et al.* (2010) Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nat. Genet.*, **42**, 790–793.
3. Chen, Y.Z., Matsushita, M.M., Robertson, P., Rieder, M., Girirajan, S., Antonacci, F., Lipe, H., Eichler, E.E., Nickerson, D.A., Bird, T.D. *et al.* (2012) Autosomal dominant familial dyskinesia and facial myokymia: single exome sequencing identifies a mutation in adenylyl cyclase 5. *Arch. Neurol.*, **69**, 630–635.
4. Cummings, C.J. and Zoghbi, H.Y. (2000) Trinucleotide repeats: mechanisms and pathophysiology. *Annu. Rev. Genomics Hum. Genet.*, **1**, 281–328.
5. Ludwig, J., Kerscher, S., Brandt, U., Pfeiffer, K., Getlawi, F., Apps, D.K. and Schagger, H. (1998) Identification and characterization of a novel 9.2-kDa membrane sector-associated protein of vacuolar proton-ATPase from chromaffin granules. *J. Biol. Chem.*, **273**, 10939–10947.
6. Nguyen, G., Delarue, F., Burckle, C., Bouzhir, L., Giller, T. and Sraer, J.D. (2002) Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J. Clin. Invest.*, **109**, 1417–1427.
7. Cruciat, C.M., Ohkawara, B., Acebron, S.P., Karaulanov, E., Reinhard, C., Ingelfinger, D., Boutros, M. and Niehrs, C. (2010) Requirement of prorenin receptor and vacuolar H+-ATPase-mediated acidification for Wnt signaling. *Science*, **327**, 459–463.
8. Ramser, J., Abidi, F.E., Burckle, C.A., Lenski, C., Toriello, H., Wen, G., Lubs, H.A., Engert, S., Stevenson, R.E., Meindl, A. *et al.* (2005) A unique exonic splice enhancer mutation in a family with X-linked mental retardation and epilepsy points to a novel role of the renin receptor. *Hum. Mol. Genet.*, **14**, 1019–1027.
9. Bernhard, S.M., Seidel, K., Schmitz, J., Klare, S., Kirsch, S., Schrezenmeier, E., Zaade, D., Meyborg, H., Goldin-Lang, P., Stawowy, P. *et al.* (2012) The (pro)renin receptor ((P)RR) can act as a repressor of Wnt signalling. *Biochem. Pharmacol.*, **84**, 1643–1650.
10. De Ferrari, G.V. and Moon, R.T. (2006) The ups and downs of Wnt signaling in prevalent neurological disorders. *Oncogene*, **25**, 7545–7553.
11. Biechele, T.L. and Moon, R.T. (2008) Assaying beta-catenin/TCF transcription with beta-catenin/TCF transcription-based reporter constructs. *Methods Mol. Biol.*, **468**, 99–110.
12. Brennan, K., Gonzalez-Sancho, J.M., Castelo-Soccio, L.A., Howe, L.R. and Brown, A.M. (2004) Truncated mutants of the putative Wnt receptor LRP6/Arrow can stabilize beta-catenin independently of Frizzled proteins. *Oncogene*, **23**, 4873–4884.
13. Kinouchi, K., Ichihara, A., Sano, M., Sun-Wada, G.H., Wada, Y., Kurauchi-Mito, A., Bokuda, K., Narita, T., Oshima, Y., Sakoda, M. *et al.* (2010) The (pro)renin receptor/ATP6AP2 is essential for vacuolar H+-ATPase assembly in murine cardiomyocytes. *Circ. Res.*, **107**, 30–34.
14. Yoshimori, T., Yamamoto, A., Moriyama, Y., Futai, M. and Tashiro, Y. (1991) Bafilomycin A1, a specific inhibitor of vacuolar-type H(+)-ATPase, inhibits acidification and protein degradation in lysosomes of cultured cells. *J. Biol. Chem.*, **266**, 17707–17712.
15. Xu, J., Feng, H.T., Wang, C., Yip, K.H., Pavlos, N., Papadimitriou, J.M., Wood, D. and Zheng, M.H. (2003) Effects of Bafilomycin A1: an inhibitor of vacuolar H(+)-ATPases on endocytosis and apoptosis in RAW cells and RAW cell-derived osteoclasts. *J. Cell. Biochem.*, **88**, 1256–1264.
16. Kimura, S., Noda, T. and Yoshimori, T. (2007) Dissection of the autophagosome maturation process by a novel reporter protein, tandem fluorescent-tagged LC3. *Autophagy*, **3**, 452–460.
17. Kuusisto, E., Kauppinen, T. and Alafuzoff, I. (2008) Use of p62/SQSTM1 antibodies for neuropathological diagnosis. *Neuropathol. Appl. Neuro.*, **34**, 169–180.
18. Hedera, P., Alvarado, D., Beydoun, A. and Fink, J.K. (2002) Novel mental retardation-epilepsy syndrome linked to Xp21.1-p11.4. *Ann. Neurol.*, **51**, 45–50.
19. Ward, A.J. and Cooper, T.A. (2010) The pathobiology of splicing. *J. Pathol.*, **220**, 152–163.
20. Lopez-Bigas, N., Audit, B., Ouzounis, C., Parra, G. and Guigo, R. (2005) Are splicing mutations the most frequent cause of hereditary disease? *FEBS Lett.*, **579**, 1900–1903.
21. Nishi, T. and Forgac, M. (2002) The vacuolar (H+)-ATPases–nature's most versatile proton pumps. *Nat. Rev. Mol. Cell. Biol.*, **3**, 94–103.
22. Marshansky, V. and Futai, M. (2008) The V-type H+-ATPase in vesicular trafficking: targeting, regulation and function. *Curr. Opin. Cell. Biol.*, **20**, 415–426.
23. Yokoyama, K. and Imamura, H. (2005) Rotation, structure, and classification of prokaryotic V-ATPase. *J. Bioenerg. Biomembr.*, **37**, 405–410.
24. Peters, C., Bayer, M.J., Buhler, S., Andersen, J.S., Mann, M. and Mayer, A. (2001) Trans-complex formation by proteolipid channels in the terminal phase of membrane fusion. *Nature*, **409**, 581–588.
25. Riediger, F., Quack, I., Qadri, F., Hartleben, B., Park, J.K., Potthoff, S.A., Sohn, D., Sihn, G., Rousselle, A., Fokuhl, V. *et al.* (2011) Prorenin receptor is essential for podocyte autophagy and survival. *J. Am. Soc. Nephrol.*, **22**, 2193–2202.
26. Contrepas, A., Walker, J., Koulakoff, A., Franek, K.J., Qadri, F., Giaume, C., Corvol, P., Schwartz, C.E. and Nguyen, G. (2009) A role of the (pro)renin receptor in neuronal cell differentiation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **297**, R250–257.
27. Dickson, D.W., Kouri, N., Murray, M.E. and Josephs, K.A. (2011) Neuropathology of frontotemporal lobar degeneration-tau (FTLD-tau). *J. Mol. Neurosci.*, **45**, 384–389.
28. Zabetian, C.P., Samii, A., Mosley, A.D., Roberts, J.W., Leis, B.C., Yearout, D., Raskind, W.H. and Griffith, A. (2005) A clinic-based study of the LRRK2 gene in Parkinson disease yields new mutations. *Neurology*, **65**, 741–744.
29. Karakoc, E., Alkan, C., O'Roak, B.J., Dennis, M.Y., Vives, L., Mark, K., Rieder, M.J., Nickerson, D.A. and Eichler, E.E. (2012) Detection of structural variants and indels within exome data. *Nat. Methods*, **9**, 176–178.
30. Benson, G. (1999) Tandem repeats finder: a program to analyze DNA sequences. *Nucleic Acids Res.*, **27**, 573–580.
31. Gelfand, Y., Rodriguez, A. and Benson, G. (2007) TRDB–the Tandem Repeats Database. *Nucleic Acids Res.*, **35**, D80–87.
32. Anderson, M.A. and Gusella, J.F. (1984) Use of cyclosporin A in establishing Epstein-Barr virus-transformed human lymphoblastoid cell lines. *In Vitro*, **20**, 856–858.
33. Desmet, F.O., Hamroun, D., Lalande, M., Collod-Beroud, G., Claustres, M. and Beroud, C. (2009) Human Splicing Finder: an online bioinformatics tool to predict splicing signals. *Nucleic Acids Res.*, **37**, e67.
34. Guthrie, C.R., Greenup, L., Leverenz, J.B. and Kraemer, B.C. (2011) MSUT2 is a determinant of susceptibility to tau neurotoxicity. *Hum. Mol. Genet.*, **20**, 1989–1999.