ИНФОРМАЦИЯ ЗА:
Наименование на заболяването
Паркинсонизъм с ранно начало и интелектуални нарушения
Определение на заболяването
Паркинсонизъм с ранно начало и интелектуални нарушения е Х-свързано неврологично заболяване характеризиращо се с забавено психомоторно развитие, интелектуално нарушение и Паркинсонова болест с ранно начало.
Четирицифрен код на заболяването по МКБ-10 (ако такъв е наличен)
G20
Код на заболяването по Orpha code
ORPHA2379
Епидемиологични данни за заболяването в Република България
Предполага се <1 / 1 000 000; Предполага се заболеваемост и болестност сходна на останалите 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. Laxova, R., Brown, E. S., Hogan, K., Hecox, K., Opitz, J. M. An X-linked recessive basal ganglia disorder with mental retardation. Am. J. Med. Genet. 21: 681-689, 1985. 3. Mata, I. F., Jang, Y., Kim, C.-H., Hanna, D. S., Dorschner, M. O., Samii, A., Agarwal, P., Roberts, J. W., Klepitskaya, O., Shprecher, D. R., Chung, K. A., Factor, S. A., and 14 others. The RAB39B p.G192R mutation causes X-linked dominant Parkinson's disease. Molec. Neurodegener. 10: 50, 2015.
Епидемиологични данни за заболяването в Европейския съюз
Предполага се <1 / 1 000 000; Неизвестни точни заболеваемост и болестност. Laxova и колеги (1985) описват три поколения в едно немско семейство. Mata и колеги (2015) съобщават за голяма американска фамилия с европейски произход, в която 7 индивида (5 мъже и 2 жени) са с Паркинсонова болест, като 2 от мъжете са и с леки интелектуални нарушения.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Laxova, R., Brown, E. S., Hogan, K., Hecox, K., Opitz, J. M. An X-linked recessive basal ganglia disorder with mental retardation. Am. J. Med. Genet. 21: 681-689, 1985. 2. Mata, I. F., Jang, Y., Kim, C.-H., Hanna, D. S., Dorschner, M. O., Samii, A., Agarwal, P., Roberts, J. W., Klepitskaya, O., Shprecher, D. R., Chung, K. A., Factor,

S. A., and 14 others. The RAB39B p.G192R mutation causes X-linked dominant Parkinson's disease. Molec. Neurodegener. 10: 50, 2015.

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): Паркинсонизъм с ранно начало и интелектуални нарушения е Х-свързано неврологично заболяване, характеризиращо се със забавено психомоторно развитие, интелектуално нарушение и Паркинсонова болест с ранно начало.

Признаците и симптомите на заболяването: Паркинсонизъм с ранно начало и интелектуални нарушения е заболяване на базалните ганглии, характеризиращо се с паркинсонизъм (постурални нарушения, тремор, ригидност), мегалоцефалия и разнообразни интелектуални дефицити. Други признаци включват персистиращ хватателен рефлекс, страбизъм и припадъци.

Етиологията и патогенезата: Wilson и колеги (2014) установяват хемизиготна мис мутация в RAB39B ген на Х-хромозома, което води до дестабилизация и повишен оборот на мутантния протеин в съответствие със загуба на функция.

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

1. Wilson, G. R., Sim, J. C. H., McLean, C., Giannandrea, M., Galea, C. A., Riseley, J. R., Stephenson, S. E. M., Fitzpatrick, E., Haas, S. A., Pope, K., Hogan, K. J., Gregg, R. G., and 21 others. Mutations in RAB39B cause X-linked intellectual disability and early-onset Parkinson disease with alpha-synuclein pathology. Am. J. Hum. Genet. 95: 729-735, 2014.

Алгоритми за диагностициране на заболяването

Алгоритми за диагностициране на заболяването: Диагноза на паркинсонизма съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест.

Анамнезата: Паркинсонизъм с ранно начало и интелектуални нарушения е заболяване на базалните ганглии, характеризиращо се с паркинсонизъм (постурални нарушения, тремор, ригидност), мегалоцефалия и разнообразни интелектуални дефицити. Други признаци включват персистиращ хватателен рефлекс, страбизъм и припадъци.

Диференциалната диагноза на заболяването: Други форми на атипичен паркинсонизъм

Лабораторни, образни и хистологични изследвания: КТ/МРТ изключва интракраниални калцификати.

Генетични изследвания и медико-генетично консултиране: Wilson и колеги (2014) установяват 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. Wilson, G. R., Sim, J. C. H., McLean, C., Giannandrea, M., Galea, C. A., Riseley, J. R., Stephenson, S. E. M., Fitzpatrick, E., Haas, S. A., Pope, K., Hogan, K. J., Gregg, R. G., and 21 others. Mutations in RAB39B cause X-linked intellectual disability and early-onset Parkinson disease with alpha-synuclein pathology. Am. J. Hum. Genet. 95: 729-735, 2014.

Алгоритми за лечение на заболяването

Алгоритми за лечение на заболяването: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест и Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.
Терапевтичните подходи към заболяването, в това число консервативни и оперативни, техните предимства, рискове и очаквана ефективност: Съобщава се за добър ефект на леводопа терапията при пациенти с това заболяване.

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. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.
3. Wilson, G. R., Sim, J. C. H., McLean, C., Giannandrea, M., Galea, C. A., Riseley, J. R., Stephenson, S. E. M., Fitzpatrick, E., Haas, S. A., Pope, K., Hogan, K. J., Gregg, R. G., and 21 others. Mutations in RAB39B cause X-linked intellectual disability and early-onset Parkinson disease with alpha-synuclein pathology. Am. J. Hum. Genet. 95: 729-735, 2014.
4. Mata, I. F., Jang, Y., Kim, C.-H., Hanna, D. S., Dorschner, M. O., Samii, A., Agarwal, P., Roberts, J. W., Klepitskaya, O., Shprecher, D. R., Chung, K. A., Factor, S. A., and 14 others. The RAB39B p.G192R mutation causes X-linked dominant Parkinson's disease. Molec. Neurodegener. 10: 50, 2015.

Алгоритми за проследяване на заболяването

Алгоритми за проследяване на заболяването: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест и Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.

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. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.

Алгоритми за рехабилитация на заболяването

Алгоритми за рехабилитация на заболяването: Съгласно Национален консенсус за диагностика и лечение на Паркинсоновата болест и Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция.

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. Национален консенсус за ранна диагностика и лечение на болестта на Алцхаймер и други форми на деменция, април 2015.

Необходими дейности за профилактика на заболяването (ако такива са приложими)

Дейности за профилактика на заболяването: Wilson и колеги (2014) установяват хемизиготна мис мутация в RAB39B ген на Х-хромозома, което води до дестабилизация и повишен оборот на мутантния протеин в съответствие със загуба на

функция. При фамилии с установен генетичен дефект би могло да се препоръча пренатална диагностика.
В т.ч. научни публикации от последните пет години и приложена библиографска справка
1. Wilson, G. R., Sim, J. C. H., McLean, C., Giannandrea, M., Galea, C. A., Riseley, J. R., Stephenson, S. E. M., Fitzpatrick, E., Haas, S. A., Pope, K., Hogan, K. J., Gregg, R. G., and 21 others. Mutations in RAB39B cause X-linked intellectual disability and early-onset Parkinson disease with alpha-synuclein pathology. Am. J. Hum. Genet. 95: 729-735, 2014.
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;
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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.
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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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MOLECULAR NEURODEGENERATION

RESEARCH ARTICLE Open Access

The *RAB39B* p.G192R mutation causes X-linked dominant Parkinson's disease

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Abstract

Objective: To identify the causal gene in a multi-incident U.S. kindred with Parkinson's disease (PD).

Methods: We characterized a family with a classical PD phenotype in which 7 individuals (5 males and 2 females) were affected with a mean age at onset of 46.1 years (range, 29-57 years). We performed whole exome sequencing on 4 affected and 1 unaffected family members. Sanger-sequencing was then used to verify and genotype all candidate variants in the remainder of the pedigree. Cultured cells transfected with wild-type or mutant constructs were used to characterize proteins of interest.

Results: We identified a missense mutation (c.574G > A; p.G192R) in the *RAB39B* gene that closely segregated with disease and exhibited X-linked dominant inheritance with reduced penetrance in females. The mutation occurred in a highly conserved amino acid residue and was not observed among 87,725 X chromosomes in the Exome Aggregation Consortium dataset. Sequencing of the *RAB39B* coding region in 587 familial PD cases yielded two additional mutations (c.428C > G [p.A143G] and c.624_626delGAG [p.R209del]) that were predicted to be deleterious *in silico* but occurred in families that were not sufficiently informative to assess segregation with disease. Experiments in PC12 and SK-N-BE(2)C cells demonstrated that p.G192R resulted in mislocalization of the mutant protein, possibly by altering the structure of the hypervariable C-terminal domain which mediates intracellular targeting.

Conclusions: Our findings implicate RAB39B, an essential regulator of vesicular-trafficking, in clinically typical PD. Further characterization of normal and aberrant RAB39B function might elucidate important mechanisms underlying neurodegeneration in PD and related disorders.

Background

Parkinson's disease (PD) is the second most common neurodegenerative disorder and though approximately 20 % of patients report a family history of the disease, kindreds that display clear Mendelian inheritance are rare. However, mutations in several genes have been shown to result in clinically typical autosomal dominant (*SNCA*, *LRRK2*, *VPS35*, *DNAJC13*) or recessive (*PARK2*, *PINK1*, *PARK7*) PD, or parkinsonism with atypical features (e.g. *PARK9*) [1]. Functional characterization of these genetic variants has provided important insights into the molecular mechanisms underlying PD and elucidated novel targets for therapeutic intervention.

Whole-exome sequencing (WES) is a powerful tool for gene discovery in pedigrees that are not sufficiently large for traditional linkage analysis [2] and this technique has been successful in identifying two causal genes for PD [3, 4]. In this study we present data from WES and *in vitro* functional analyses that demonstrate that a missense mutation (p.G192R) in the *RAB39B* gene is the causative variant in a multi-incident family with clinically typical PD.

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Results

We studied a U.S. family of European origin in which 7 individuals (5 males and 2 females) were affected and met UK PD Society Brain Bank clinical diagnostic criteria for PD [5] (Fig. 1a). The clinical characteristics of the affected family members are provided in Table 1. None of these individuals displayed atypical findings on neurological examination but two of them (III-15 and IV-4) had mild intellectual disability since childhood. DNA was available for 6 affected and 10 unaffected members of the family. We performed WES on 4 affected (III-4, III-9, III-11, and III-18) and 1 unaffected (III-13) family members. We filtered out all variants with a frequency >1 % in 515 controls from the NHLBI Exome Sequencing Project [6, 7] or that failed to meet the quality thresholds of the Genome Analysis ToolKit (GATK) "Best Practices" [8]. We identified three nonsynonymous variants that passed all filters, segregated with PD among the 5 individuals who underwent WES, and were confirmed by Sanger sequencing: *USP1* c.573G > A (p.M191I), *MVP* c.2594G > T (p.G865V), and *RAB39B* c.574G > A (p.G192R) (Table 2). We then genotyped these three variants in all remaining family members and found that only *RAB39B* p.G192R was present in all six affected subjects. Furthermore, *RAB39B* p.G192R was not observed among 87,725 X chromosomes successfully sequenced for *RAB39B* in the Exome Aggregation Consortium database (ExAC; http://exac.broadinstitute.org). The amino acid G192 is highly conserved across species (Fig. 1b) and this mutation is predicted to be deleterious as evidenced by a Combined Annotation Dependent Depletion (CADD) [9] score of 29.4.

We then screened for *RAB39B* p.G192R in 2 cohorts of PD patients from the Parkinson's Genetic Research Study (PaGeR). Cohort I was comprised of 203 "multiplex" families ascertained from across the U.S. (mean age at onset of probands, 57.3 years; male, 61.9 %) in which at least 2 individuals with PD were enrolled. Cohort II included 1298 unrelated PD patients (mean age at onset, 59.1 years; male, 69.2 %) enrolled primarily at movement disorder clinics in the Pacific Northwest regardless of family history. *RAB39B* p.G192R was not found in any other PD patients across PaGeR cohorts I and II (total n = 1501). We also sequenced the entire *RAB39B* coding region in the subset of patients from both PaGeR cohorts (n = 587) who reported a family history of PD and discovered two additional mutations, c.428C > G (p.A143G) and c.624_626delGAG (p.R209del). Neither

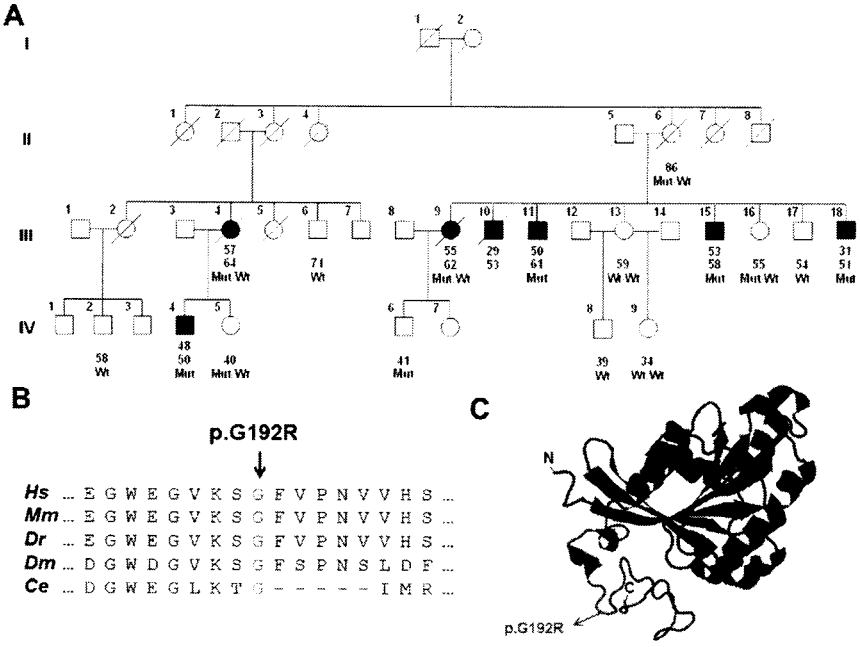

Fig. 1 Identification of the *RAB39B* p.G192R mutation by whole exome sequencing in a multigenerational kindred with Parkinson's disease. **a** Pedigree diagram; individuals affected with Parkinson's disease are represented with black symbols, unaffected individuals with open symbols. Age at onset is indicated immediately below each symbol, followed by age at last clinical evaluation. Wt = wild type; Mut = mutation (p.G192R). **b** Multispecies protein sequence alignment. *Hs* = *Homo sapiens*; *Mm* = *Mus musculus*; *Dr* = *Danio rerio* (zebrafish); *Dm* = *Drosophila melanogaster*; *Ce* = *Caenorhabditis elegans*. **c** Predicted protein structure of RAB39B using Protein Homology/analogY Recognition Engine V 2.0 (Phyre²; http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) [23]; 89 % of the amino acid residues were modeled at >90 % confidence. P.G192R is located in the hypervariable C-terminal domain which is depicted in red

Table 1 Clinical characteristics of the affected members of the pedigree

Characteristic	Patient						
	III-4	III-9	III-10	III-11	III-15	III-18	IV-4
Sex	F	F	M	M	M	M	M
Age at onset, yr	57	55	29	50	53	31	48
Age at last evaluation, yr	64	62	53	61	58	51	50
Age at death, yr	70	64	56	—	—	—	—
Bradykinesia	Y	Y	Y	Y	Y	Y	Y
Rigidity	Y	Y	Y	Y	Y	Y	Y
Resting tremor	Y	N	Y	Y	Y	Y	Y
Postural instability	Y	N	Y	Y	N	Y	Y
Unilateral onset	Y	Y	Y	Y	Y	Y	Y
Levodopa response	Y	Y	Y	Y	Y	Y	NT
Levodopa-induced dyskinesia	N	Y	Y	Y	N	Y	—
Mild, lifelong intellectual disability	N	N	N	N	Y	N	Y
Hoehn and Yahr stage[a]	4	3	4	2.5	2	5	2.5
MDS-UPDRS III score[a]	NA	24	NA	26	19	52	11

MDS-UPDRS III = Movement Disorder Society Unified Parkinson's Disease Rating Scale Part III; NA = not available; NT = no trial
[a]Determined at last evaluation

of these mutations were present in the ExAC dataset and both are predicted to be deleterious *in silico* with CADD scores of 21.8 (p.A143G) and 20.4 (p.R209del). Each mutation was present in a single family but neither of the two pedigrees was sufficiently informative to assess segregation with disease (Additional file 1: Figure S1).

We then investigated the effects of *RAB39B* p.G192R *in vitro*. In PC12 and SK-N-BE(2)C cells transfected with mutant and wild-type constructs there was no substantial difference in RAB39B protein expression (Figs. 2a and 3a). In NGF-differentiated PC12 cells wild-type RAB39B protein was visualized throughout the cytoplasm of cell bodies and neuritic processes (Fig. 2b), and co-localized with the vesicular marker chromogranin A. However, mutant (p.G192R) RAB39B was largely restricted to cell bodies with negligible amounts of protein evident in neuritic processes. In these experiments there was robust expression of both mutant and wild type RAB39B protein, but cellular phenotype can sometimes differ based on the level of transgene over-expression [10]. Thus we used an alternate vector and method of transfection to over-express RAB39B at lower levels in retinoic acid-differentiated SK-N-BE(2)C cells. Wild type RAB39B protein was frequently visualized within the cytoplasm and at the plasma membrane (co-localized with EGFR; Fig. 3b). However, while mutant RAB39B protein was also abundant in the cytoplasm, it was less frequently observed at the plasma membrane. To quantify these findings we performed immunoblot analysis of fractionated protein extracts from these cells (Fig. 3c). The proportion of membrane-bound to cytosolic RAB39B protein was significantly lower in cells expressing mutant protein than wild type protein ($p < 0.01$).

Discussion

In the present study we provide strong evidence that the missense mutation p.G192R in *RAB39B* results in clinically typical, levodopa responsive PD. The pattern of inheritance is X-linked dominant with reduced penetrance among females. Age at onset varied by nearly three decades and was lower in males. A single male mutation

Table 2 Candidate variants identified by whole exome sequencing

Gene	Position (hg19)	Transcript	dbSNP	Variation		Allele frequency (%)[a]	CADD score	Segregation[b]
				Nucleotide	Amino acid			
USP1	Chr1:62910424	NM_001017415.1	—	c.573G > A	p.M191I	0	27.1	5/6
MVP	Chr16:29859222	NM_005115.4	rs151174471	c.2594G > T	p.G865V	0.07	7.19	5/6
RAB39B	ChrX:154490156	NM_171998.2	—	c.574G > A	p.G192R	0	29.4	6/6

CADD Combined Annotation Dependent Depletion
[a]Frequency among chromosomes successfully sequenced for *USP1* (n = 111,418), *MVP* (n = 121,248), and *RAB39B* (n = 87,725) in the Exome Aggregation Consortium database (http://exac.broadinstitute.org)
[b]Number of affected individuals with the variant/total number of affected individuals with genotypes in the pedigree

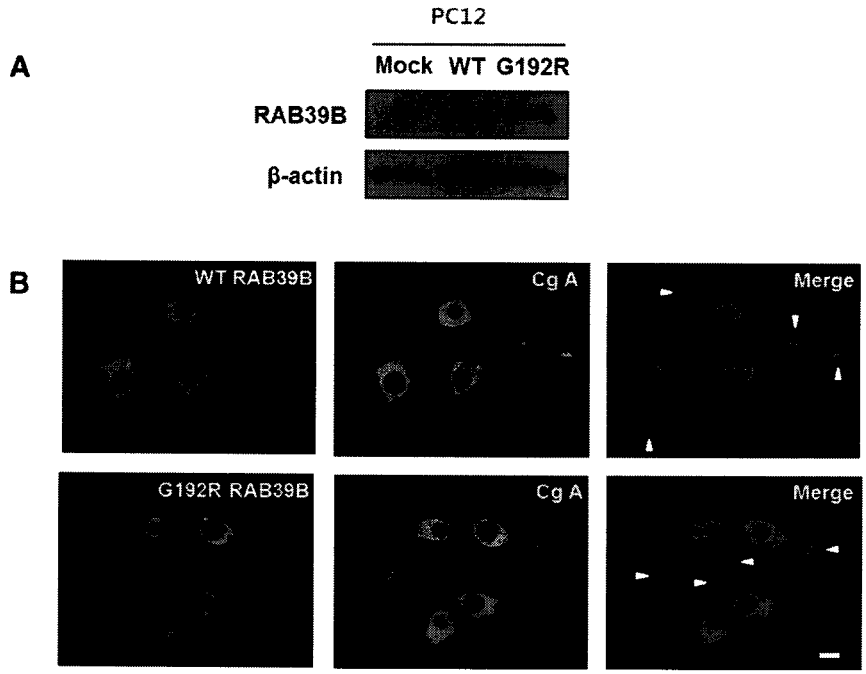

Fig. 2 Effect of p.G192R on RAB39B expression and localization in PC12 cells. **a** Western blot of rat pheochromocytoma (PC12) cells transfected with a vector that was empty (mock), or contained a wild-type or mutant (p.G192R) construct. There was no difference in protein expression between wild-type and mutant RAB39B. **b** Immunofluorescent microscopy of nerve growth factor-differentiated PC12 cells transfected with wild-type or mutant (p.G192R) myc-tagged RAB39B. The wild-type protein is visualized throughout the cell bodies and neuritic processes, together with the vesicular marker chromogranin A, whereas the mutant protein is largely confined to cell bodies. Arrowheads indicate terminal regions of neurites. The scale bar corresponds to 10 μm

carrier was unaffected at age 41. Since his current age is below the age at onset observed for 3 of the 5 affected males in the pedigree, he might well become symptomatic over time. Alternatively, it is possible that the presence of other genetic or environmental factors are necessary for p.G192R to achieve full penetrance.

RAB39B was first linked to human disease in 2010 when a nonsense and a splice site mutation in the gene were shown to cause mental retardation, sometimes accompanied by epilepsy and autism spectrum disorder, in the male members of two families [11]. Subsequently, duplications and triplications of a genomic region containing *RAB39B* were discovered in males from three families with a similar phenotype [12]. Recently, two families were reported with early onset parkinsonism in males and a missense mutation (p.T168K) or a complete deletion of *RAB39B* [13]. An autopsy of one such subject showed dopaminergic neuron loss in the substantia nigra and widespread Lewy body pathology. However, affected individuals all had features that were atypical for PD including intellectual disability, macrocephaly, and in the majority of cases, a lack of response to levodopa. In contrast, most individuals in our family displayed a classical PD phenotype, with the exception of two affected males who had mild intellectual disability, and both males and females were affected.

The explanation for the wide range of phenotypes associated with *RAB39B* mutations is not entirely clear. Previously reported mutations result in either overexpression of wild-type protein [12] or a complete loss of protein expression [11, 13]. However, the mutation discovered in our family (p.G192R) did not substantially change the overall amount of RAB39B protein expressed in PC12 or SK-N-BE(2)C cells. Instead, p.G192R appears to alter intracellular localization as the mutant protein did not properly migrate to the neuritic processes of NGF-differentiated PC12 cells and to the plasma membrane in SK-N-BE(2)C cells. This is consistent with the structural location of p.G192R based on current knowledge of Rab proteins. RAB39B is one of over 60 members of the human Rab GTPase family [14]. Rab GTPases act as molecular switches, cycling between active (GTP-bound) and inactive (GDP-bound) states to regulate intracellular vesicular trafficking in a temporally and spatially sensitive manner [15]. The C-terminus of Rab proteins contains a hypervariable domain (HVD) of 35-40 amino acids which through interactions with effector proteins plays a major role in targeting each Rab to the appropriate

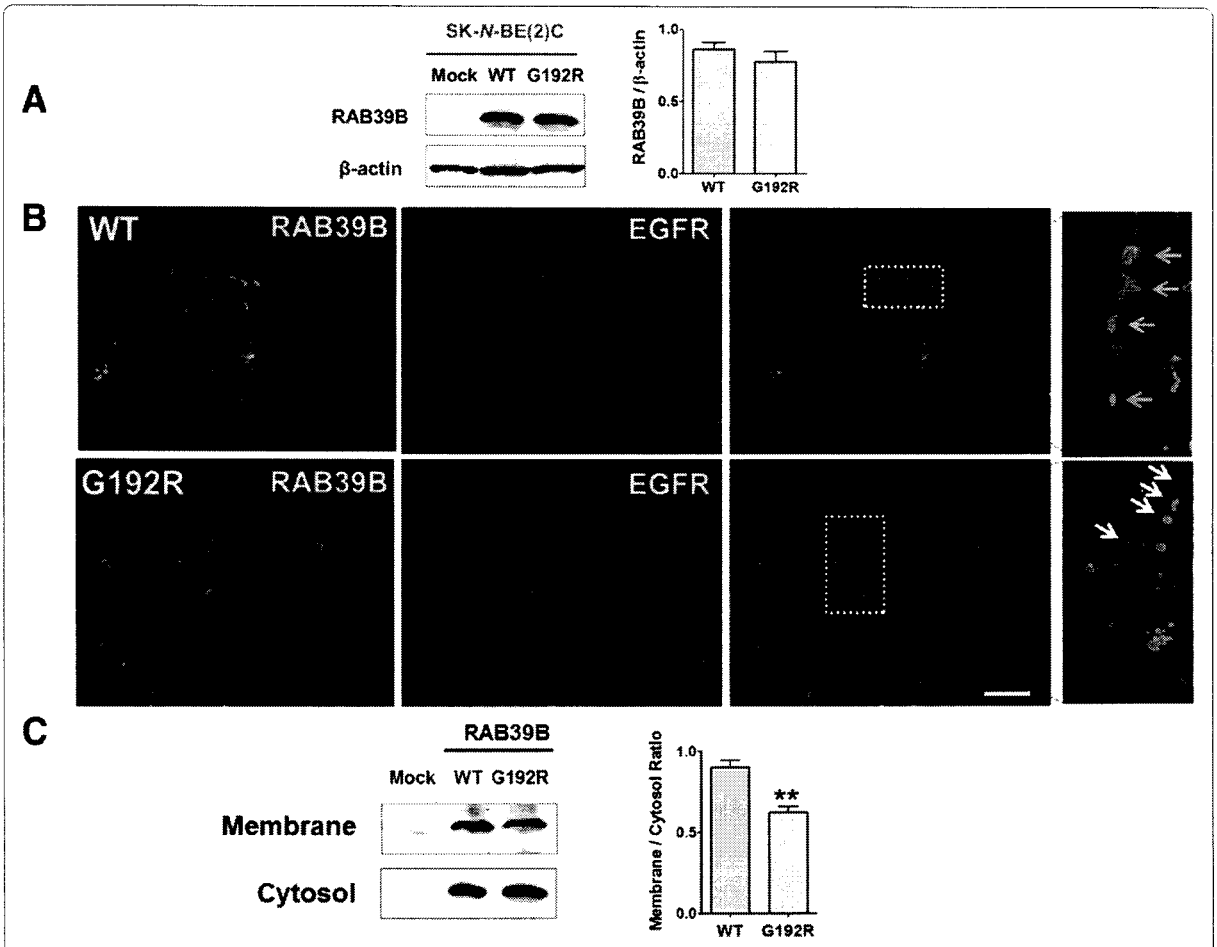

Fig. 3 Effect of p.G192R on RAB39B expression and localization in SK-N-BE(2)C cells. **a** Western blot of human neuroblastoma (SK-N-BE(2)C) cells transfected with a vector that was empty (mock), or contained a wild-type or mutant (p.G192R) construct. There was no significant difference in protein expression between wild-type and mutant RAB39B (Student's *t*-test, $p > 0.05$. **b** Immunofluorescent microscopy of retinoic acid-differentiated SK-N-BE(2)C cells transfected with wild-type or mutant (p.G192R) GFP-tagged RAB39B. Wild-type protein was seen within the cytoplasm and at the plasma membrane where it co-localized with epidermal growth factor receptor (EGFR; blue arrows). Although mutant RAB39B protein was also apparent in the cytoplasm, it less often co-localized with EGFR (white arrows). The scale bar corresponds to 10 μm. **c** Immunoblot analysis of fractionated protein extracts performed on SK-N-BE(2)C cells transfected with a vector that was empty (mock), or contained GFP-tagged wild-type or mutant RAB39B. Double asterisks indicate a significant difference ($p < 0.01$) by Student's *t*-test ($n = 3$ replicates)

intracellular location [14]. The HVD has a high content of helix-breaking proline and glycine residues which contribute to the extended structure that allows for necessary protein interactions [16]. Because p.G192R is located within the HVD (Fig. 1c) and eliminates one such glycine moiety the mutation might disrupt proper targeting of RAB39B by inhibiting binding to effector molecules. Furthermore, the fact that two heterozygous females in our pedigree were affected raises the possibility that p.G192R might exert its pathogenic effects through a dominant negative mechanism. Interestingly, a dominant negative mutation in another Rab protein (Rab8) has been described that when introduced into *Xenopus laevis* induces retinal degeneration and shifts localization of the protein from Golgi and post-Golgi membranes to the cytoplasm [17].

Though many Rab proteins are well studied, the precise localization and function of RAB39B are just beginning to emerge. RAB39B is neuron-specific and plays a role in synapse formation and maintenance [11]. Recent evidence suggests that one function of RAB39B, through its interaction with protein interacting with C-kinase 1 (PICK1), is to regulate the subunit composition of heterotetrameric AMPA receptors [18]. In the absence of RAB39B, AMPA receptor composition shifts towards non GluA2-containing $Ca2^{+}$-permeable forms. The resulting alteration in synaptic activity has been posited to underlie the lifelong intellectual disability and

behavioral problems seen with loss-of-function mutations in *RAB39B*. However, the mechanism by which dysregulation of *RAB39B* leads to the selective neurodegenerative changes seen in PD is not yet known. Our discovery that p.G192R results in a "pure" PD phenotype provides an opportunity to address this important question by examining the effects of this mutation in model systems in future studies.

Conclusions

Our findings implicate RAB39B, an essential regulator of vesicular-trafficking, in clinically typical PD. Loss-of-function mutations in this gene were previously shown to cause X-linked recessive mental retardation sometimes accompanied by autism spectrum disorder, and members of two such families were later shown to develop atypical parkinsonism. However, the phenotype in our family is classical, levodopa-responsive PD and both males and females are affected. We present *in vitro* data that provide a potential explanation for the substantial difference in phenotype between our family and those reported elsewhere. Unlike previously reported mutations which result in a complete loss of protein expression, the mutation that we have discovered (p.G192R) does not alter the overall amount of protein expressed but rather its intracellular localization. Our results suggest that dysregulation of RAB39B, which is thought to mediate vesicular transport, can lead to selective neurodegenerative changes in the absence of lifelong cognitive/behavioral dysfunction, and have important implications for future research.

Methods

Exome sequencing

The exome was captured using the SeqCap EZ Exome v3.0 kit (Roche/Nimblegen, Madison, WI) and sequenced with 100-base pair (bp) paired-end reads on a HiSeq2500 (Illumina, San Diego, CA) to achieve a mean coverage of 80-100X. Sequence reads were mapped to the human reference genome (GRCh37) using the Burrows-Wheeler Aligner. Variants were jointly called using the GATK HaplotypeCaller following the developer's recommended best practices [8] (https://www.broadinstitute.org/gatk/guide/best-practices) and annotated with SnpEff [19] based on the RefSeq gene set (http://www.ncbi.nlm.nih.gov/refseq). We flagged variants failing to meet the quality thresholds described by the GATK "Best Practices" of QD (Quality by Depth) < 2.0, FS (FisherStrand; Fisher's exact test for strand bias) > 60.0, MQ (Mapping Quality; overall mapping quality of reads averaged over all samples) < 40.0, HaplotypeScore (probability that reads flanking a variant can be explained by ≤ 2 haplotypes) > 13.0, MQRankSum (comparison of mapping qualities of reads for reference versus alternate allele) < -12.5, and ReadPosRankSum (measure of bias in position within reads between reference and alternate allele) < -8.0. We excluded alleles that occurred at a frequency >1 % in 515 unrelated white controls selected from the NHLBI Exome Sequencing Project [6, 7]. Finally, we used custom software to analyze variants that passed all filters to identify alleles that segregated with disease.

Sanger sequencing and genotyping

Sanger sequencing was used to confirm and genotype candidate variants in all available members of the pedigree. We also sequenced the entire *RAB39B* coding region and intron-exon boundaries in the probands from PaGeR Cohort I (n = 203) and in the subset of patients from Cohort II (n = 384) who reported a family history of PD. Sequencing was performed using the Applied Biosystems Big-Dye Terminator v3.1 Cycle Sequencing Kit on an ABI PRISM 3130 genetic analyzer (Applied Biosystems, Foster City, CA) as described elsewhere [20]. Sequence data were base-called, aligned, and scanned for variation using Mutation Surveyor (SoftGenetics, State College, PA). *RAB39B* p.G192R was genotyped in the remainder of PaGeR Cohort II using a custom TaqMan assay.

Protein expression assays

Two myc-tagged constructs encoding either wild type or mutant (p.G192R) RAB39B protein were created using the vector pcDNA 3.1/myc-His (Invitrogen Life Technologies, Carlsbad, CA). Rat pheochromocytoma (PC12) and human neuroblastoma (SK-N-BE(2)C) cells were grown and transfected with wild type or mutant constructs using either a retrovirus (pCL Vector System, Orbigen, San Diego, CA) for PC12 cells or Lipofectamine (Invitrogen) for SK-N-BE(2)C cells using previously described methods [21]. After 24 h the cells were lysed, and the lysates were subjected to 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, CA). The membranes were incubated with an anti-myc antibody (Roche Life Sciences, Branford, CT) and detection was achieved using the Novex ECL chemiluminescent substrate reagent kit (Invitrogen).

Protein trafficking experiments

PC12 cells were transfected with a wild type or mutant (p.G192R) myc-tagged *RAB39B* construct as described in the previous section and differentiated with nerve growth factor (NGF) for 4 days. The cells were fixed in 4 % paraformaldehyde and then incubated overnight at 4 °C with rabbit anti-myc and anti-chromogranin A antibodies. SK-N-BE(2)C cells were transfected with GFP-tagged wild type or mutant *RAB39B* constructs (pEGFP-N1 vector; Clontech Laboratories, Mountain View, CA) using Lipofectamine. After retinoic acid-induced differentiation for 4 days the cells were fixed in 4 % paraformaldehyde and

incubated overnight at 4 °C with anti-epidermal growth factor receptor (anti-EGFR) antibody (a plasma membrane marker; Cell Signaling Technology, Danvers, MA) using methods described elsewhere [22]. Hoechst 33342 was used for counterstaining and confocal analysis was performed using an Olympus IX81 microscope.

Subcellular fractionation

SK-N-BE(2)C cells were transfected with GFP-tagged wild type or mutant *RAB39B* constructs as described in the previous section. After 24 h the cells were lysed and membrane and cytoplasmic fractions were prepared using the Subcellular Protein Fractionation kit for Cultured Cells (Thermo Scientific, Rockford, IL) according to the manufacturer's recommendations. The fractionated lysates were subjected to 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride membrane. The membranes were incubated with an anti-GFP antibody (Santa Cruz Biotechnology). Images of western blots were captured using the ChemiDoc XRS system (Bio-Rad Laboratories) and scanned films were quantified using Image J software (http://rsb.info.nih.gov/ij/).

Additional file

Additional file 1: Figure S1. Pedigrees with variants of unknown significance. Pedigrees in which the RAB39B (A) c.428C>G (p.A143G) and (B) c.624_626delGAG (p.R209del) variants were observed. Individuals affected with Parkinson's disease are represented with black symbols, unaffected individuals with open symbols. Age at onset is indicated immediately below each symbol, followed by age at last clinical evaluation. Wt = wild type; Mut = mutation. (PDF 35 kb)

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

IFM and CPZ had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: IFM, CPZ. Acquisition of data: YJ, C-HK, DSH, MOD, AS, PA, JWR, OK, DRS, KAC, SAF, AJE, FJR, DSH, IL, JBL, DY, MI-M, EM, TT, BAC, S-CH, K-SK. Analysis and interpretation of data: IFM, YJ, C-HK, DSH, MOD, KLE, K-E, CPZ. Drafting of the manuscript: IFM, CPZ. Critical revision of the manuscript for important intellectual content: YJ, C-HK, DSH, MOD, AS, PA, JWR, OK, DRS, KAC, SAF, AJE, FJR, DSH, IL, JBL, DY, MI-M, EM, TT, BAC, S-CH, KLE, K-SK. All authors read and approved the final manuscript.

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Mutations in *RAB39B* Cause X-Linked Intellectual Disability and Early-Onset Parkinson Disease with α-Synuclein Pathology

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Advances in understanding the etiology of Parkinson disease have been driven by the identification of causative mutations in families. Genetic analysis of an Australian family with three males displaying clinical features of early-onset parkinsonism and intellectual disability identified a ~45 kb deletion resulting in the complete loss of *RAB39B*. We subsequently identified a missense mutation (c.503C>A [p.Thr168Lys]) in *RAB39B* in an unrelated Wisconsin kindred affected by a similar clinical phenotype. In silico and in vitro studies demonstrated that the mutation destabilized the protein, consistent with loss of function. In vitro small-hairpin-RNA-mediated knockdown of *Rab39b* resulted in a reduction in the density of α-synuclein immunoreactive puncta in dendritic processes of cultured neurons. In addition, in multiple cell models, we demonstrated that knockdown of *Rab39b* was associated with reduced steady-state levels of α-synuclein. Post mortem studies demonstrated that loss of RAB39B resulted in pathologically confirmed Parkinson disease. There was extensive dopaminergic neuron loss in the substantia nigra and widespread classic Lewy body pathology. Additional pathological features included cortical Lewy bodies, brain iron accumulation, tau immunoreactivity, and axonal spheroids. Overall, we have shown that loss-of-function mutations in *RAB39B* cause intellectual disability and pathologically confirmed early-onset Parkinson disease. The loss of RAB39B results in dysregulation of α-synuclein homeostasis and a spectrum of neuropathological features that implicate RAB39B in the pathogenesis of Parkinson disease and potentially other neurodegenerative disorders.

Parkinsonism is a neurological syndrome characterized by tremor, rigidity, balance problems, and a slowing of movement. The most common cause of parkinsonism is Parkinson disease (PD [MIM 168600]), which accounts for up to 70% of this syndrome. PD is a common progressive neurodegenerative disorder with motor symptoms due to the death of dopamine-generating cells, predominantly in the substantia nigra (SN). The pathological hallmark of PD is accumulation of α-synuclein in Lewy bodies and Lewy neurites, although additional pathology (such as neurofibrillary tangles [NFTs]) can be observed.[1] Recent genetic studies have driven advances in understanding the molecular pathogenesis of PD, and preclinical discovery projects have investigated compounds that target the identified proteins as a precursor to etiology-based therapeutics. To date, 18 PD-associated loci have been reported, and variants in 13 monogenic or susceptibility genes have been identified.[2] Common pathogenic mechanisms associated with these genes include protein turnover, mitochondrial function, and oxidative-stress pathways. However, approximately 90% of individuals with PD do not have a defined genetic etiology. Variants in known genes account for ~10% of the variation in PD liability, suggesting that variants in additional genes and susceptibility loci remain to be identified.[3,4]

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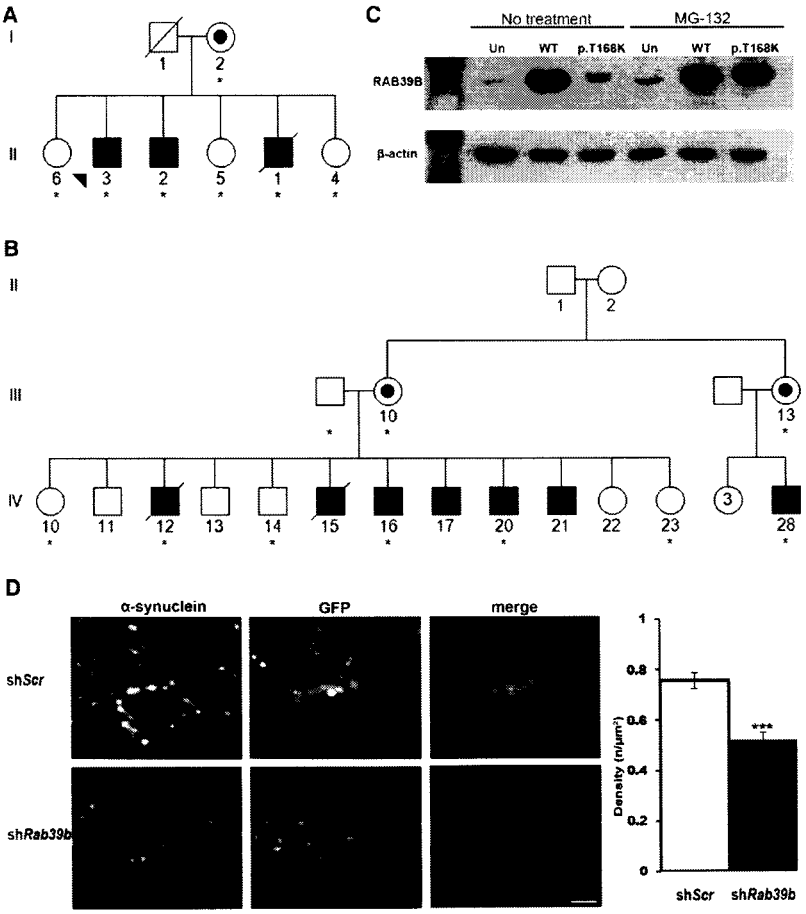

Figure 1. Identification of Mutations in *RAB39B*

(A and B) Simplified pedigree structure of the Australian (A) and Wisconsin (B) kindreds. Asterisks indicate DNA samples analyzed.

(C) Immunoblot analysis (12162-1-AP, Proteintech; 1:1,000) of RAB39B in BE(2)-M17 neuroblastoma cells grown in the absence or presence of 10 μM MG-132. Abbreviations are as follows: Un, untransfected; WT, wild-type; and p.T168K, RAB39B p.Thr168Lys. β-actin was used for confirming equivalent protein loading (A5441, Sigma-Aldrich; 1:5,000).

(D) Representative images of mouse hippocampal neurons transduced with lentiviral vectors encoding GFP and either scramble (sh*Scr*) or sh*Rab39b* sequences. Images were captured, and Z-space slices (0.3–0.4 μm) were deconvolved and flattened by maximum projection. ImageJ analysis software (the "Gran filter" plug-in set the size from 1 to infinity) was used to measure α-synuclein density relative to the area of infected dendrites. Quantification showed a significant reduction (0.52 ± 0.03 versus 0.76 ± 0.03, mean ± SEM, $p \leq 0.0005$) in the density of α-synuclein immunoreactive puncta in neurons downregulated for *Rab39b*. The experiment was performed in triplicate (n = 42 neurons scored). The scale bar represents 20 μm.

We identified an Australian kindred with three brothers who presented in childhood with nonprogressive intellectual disability (ID), which included delayed developmental milestones, cognitive impairment, and macrocephaly (Figure 1; Table S1, available online). Subsequently, early-onset parkinsonism (onset prior to 45 years of age) was also apparent, although the clinical progression and presentation varied. The proband developed tremor in late childhood, but the symptoms did not progress to frank parkinsonism. In contrast, his male siblings developed tremor from their late 30s and were diagnosed with L-DOPA-responsive akinetic-rigid PD by their mid-40s. A complete description of the phenotype is presented in Table S1. We collected samples from the Australian family after receiving institutional ethics approval from Royal Childrens Hospital (Melbourne) and written informed consent from participants. Genomic DNA was isolated from whole blood, and primary fibroblast cultures were generated according to standard protocols. SNP array and linkage analysis using a recessive homozygous model did not demonstrate linkage to the autosomes but did identify two ~10.6 Mb haplotypes shared by the affected brothers at Xp22.2 and Xq27.3–qter (chrX: 3,624,034–14,291,092 and chrX: 145,644,895–tel, respectively; GRCh38/hg38, UCSC Genome Browser; Table S2). Copy-number variation and subsequent PCR analysis identified a ~45 kb deletion within the Xq haplotype (ClinVar accession number SCV00019029). The deletion segregated with the disease and resulted in the complete deletion of *RAB39B* (RAB39B, member RAS oncogene family [MIM 300774]) and the last three coding exons of *CLIC2* (chloride intracellular channel 2 [MIM 300138]). To assess *RAB39B* expression, we extracted total RNA from fibroblasts by using the SV Total RNA Isolation System (Promega) and synthesized cDNA with the Transcriptor First Strand cDNA Synthesis Kit (Roche). Consistent with the genomic data, the *RAB39B* and *CLIC2* transcripts were not detected by RT-PCR analysis of fibroblast cells derived from affected individuals (Figure S1).

The phenotype of the Australian kindred is similar to the basal ganglia disorder (Waisman syndrome [MIM 311510]) reported for a Wisconsin kindred[5] (Figure 1; Table S1). Members of the defining family included 13 affected males who presented with variable degrees of ID and early-onset parkinsonism. Multipoint linkage analysis of the Wisconsin family previously localized the disease-causing mutation to Xq27.3–qter with a maximum multipoint LOD of 6.75 at the genetic marker F8C (chrX:154,929,351–154,929,630; GRCh38/hg38). The minimal Xq linkage region in the Australian kindred is within the Xq27.3–qter interval and therefore defines the shared critical linkage interval.[5]

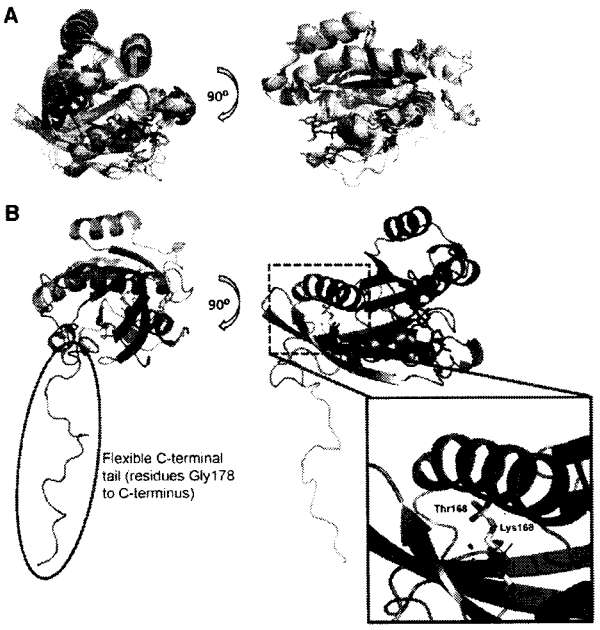

Figure 2. In Silico Modeling of the RAB39B Structure Predicts that p.Thr168Lys Is Destabilizing

(A) We generated a molecular model of the RAB39B structure (blue) by superimposing the structures of several known RAB proteins (gray) available in the Protein Data Bank (YTP1 [PDB 2BCG], RAB1A [PDB 3TKL], RAB11B [PDB 2F9L], RAB18 [PDB 1X3S], RAB23 [PDB 1Z2A], and RAB30 [PDB 2EW1]). GTP (green sticks) and Mg^{2+} (magenta sphere) are from the structure of RAB5A after superposition.

(B) We then used the predicted RAB39B structure (blue) to model the position of the native (Thr168, red) and altered (Lys168, yellow) amino acid. In the native form, Thr168 is predicted to interact with Leu60 within the interswitch region of RAB39B. The internal placement of the large positively charged residue in the p.Thr168Lys protein is predicted to destabilize the protein. The quality of the model was tested with PROCHECK,[12] ANOLEA,[13] and Verify3D.[14]

Genomic DNA from individuals of the Wisconsin kindred was kindly provided by Professor Ronald Gregg. Ethics approval was provided by the institutional review board (IRB) at the University of Wisconsin, and informed consent was obtained. Direct sequencing of *RAB39B* identified a missense mutation (c.503C>A [p.Thr168Lys]; RefSeq accession number NM_171998.2, ClinVar SCV000190018) that segregated with disease (Figure S2) and was not detected in 200 unrelated control individuals or public databases (dbSNP137 and ESP6500). The threonine residue is conserved in evolution (Figure S3), and the mutation is predicted to be damaging by PolyPhen-2 and SIFT. In contrast, no sequence variants were observed by direct sequencing of *CLIC2* in the Wisconsin kindred.

The absence of mutations in *CLIC2* in the Wisconsin kindred suggests that disruption of *RAB39B* is the cause of the shared phenotype in both families. It is possible that deletion of *CLIC2* in the Australian kindred might act as a disease modifier, although the phenotypic features (seizures and cardiac anomalies) associated with a missense mutation in *CLIC2* were not observed.[7] X chromosome exome sequencing of affected males from both kindreds confirmed the deleterious *RAB39B* changes and did not identify any other candidate variants within the linkage regions (Table S3).

In silico modeling of RAB39B was performed with the structure prediction programs MODELER[8] and HHpred,[9] and protein structures were visualized and superimposed with PyMOL. This analysis suggested that Thr168 is buried within the wild-type protein and interacts with Leu60 in the interswitch region, which undergoes conformational changes upon GTP-GDP exchange[10,11] (Figure 2). The mutation introduces a large, positively charged lysine residue that is predicted by multiple algorithms, including ERIS[15] and PoPMuSiC,[16] to destabilize the protein. We could not directly test this because endogenous RAB39B was not detectable in fibroblast cells; therefore, we generated stable BE(2)-M17 neuroblastoma lines overexpressing wild-type RAB39B and altered (p.Thr168 Lys) RAB39B. The complete *RAB39B* open reading frame was amplified from human brain cDNA and cloned into the mammalian expression vector pcDNA3.1 (Invitrogen). We used site-directed mutagenesis (QuickChangeII) to generate the p.Thr168Lys altered RAB39B construct and Sanger sequenced all clones to verify that no additional variants were present. RT-PCR analysis confirmed similar expression of the wild-type and altered constructs (data not shown). In contrast, immunoblot analysis revealed high steady-state levels of exogenous wild-type RAB39B but very low levels of exogenous altered RAB39B. Immunoblot and immunofluorescence analysis of the cells after treatment with the proteasome inhibitor MG-132 confirmed that the reduced steady-state level of altered RAB39B was due to rapid turnover of the protein by the ubiquitin proteasome system (Figure 1; Figure S4). These results confirm the in silico modeling suggesting that the altered protein is destabilized and collectively demonstrate that loss of function of RAB39B causes ID and parkinsonism. Previous studies have associated *RAB39B* mutations with ID[17–19] (pedigrees D-23 and MRX72; Table S1). The absence of parkinsonism in these additional families could be due to the individuals' age at reporting, given that our data suggest that parkinsonism is likely to manifest after the second decade, albeit with some variability in both onset and clinical severity (Table S1). However, the lack of clinical data and inability to re-examine affected individuals mean that it is difficult to determine whether the phenotype associated with loss of RAB39B function represents an age-dependent progression of ID and parkinsonism or a spectrum of heterogeneous phenotypes extending from ID to ID with parkinsonism (see below).

Rab GTPases belong to the Ras superfamily of small GTPases and act as essential regulators of vesicular trafficking. They dynamically localize to distinct intracellular membranes and regulate vesicular transport by recruiting effector proteins.[20] The precise localization and function of RAB39B is unknown, but the protein is thought to play a role in synapse formation and maintenance.[17,18,21]

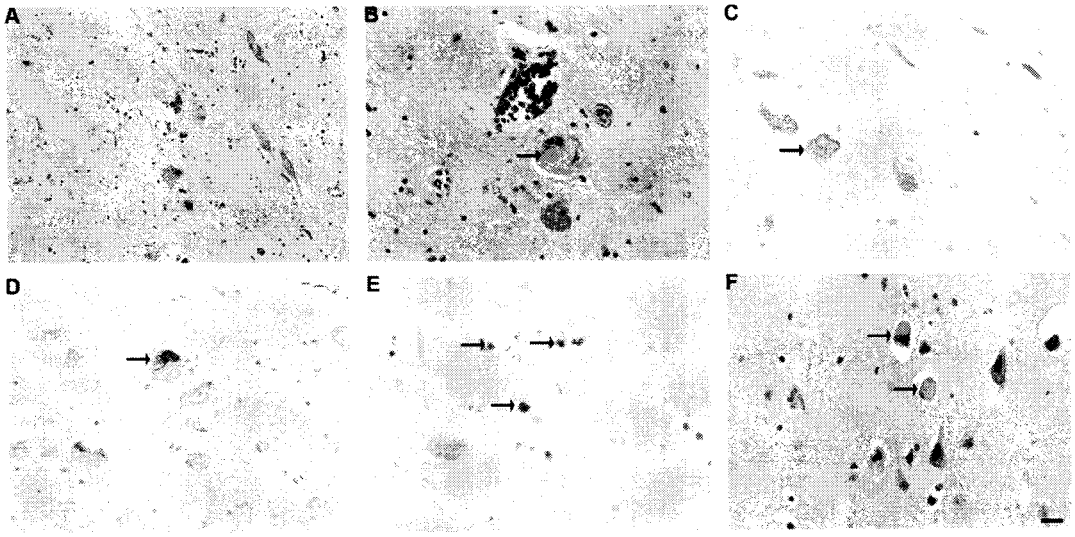

Figure 3. Neuropathology Associated with Loss of RAB39B
CNS postmortem tissue from II:1 was collected at autopsy, and microscopic examination was performed on H&E-stained sections. To detect PD-associated proteins, we applied immunohistochemistry to 5 μm formalin-fixed paraffin-embedded sections as previously described.[25] Primary antibodies utilized were anti-α-synuclein (97/8; 1:1,000)[23] and anti-tau (A0024, Dako; 1:500). CNS nonhaem iron (Fe^{2+} and Fe^{3+}) was detected with a previously described Perl's methodology.[26] The scale bar represents 50 μm (A) or 20 μm (B–F). (A–E) SN sections show (A) neuronal loss and pigment incontinence (H&E), (B) intraneuronal Lewy bodies (H&E), (C) α-synuclein-reactive Lewy bodies and neurites, (D) tau-immunoreactive intraneuronal NFTs, and (E) extracellular iron deposition (Perl's stain). (F) Neocortical Lewy bodies were identified by H&E staining.

In support of this, we observed colocalization of endogenous RAB39B with markers of the vesicular-transport pathway, particularly the early endosome in mouse and human neuroblastoma cells (Figure S5). Given the postmortem results (below) and the association between α-synuclein and vesicular-trafficking pathways,[22] we tested the effect of downregulation of RAB39B on α-synuclein localization. Mouse hippocampal neurons were prepared and transduced with lentivirus expressing validated *Rab39b* small hairpin RNA (shRNA) as previously described.[17] Fourteen days after transduction, we observed that the density of α-synuclein immunoreactive puncta in the dendritic processes was 30% lower than in the cells transduced with the scramble control shRNA ($p \leq 0.0005$; Figure 1; Figure S6). Immunoblot analysis confirmed ~40% reduction of RAB39B but also demonstrated that α-synuclein levels (detected with the anti-α-synuclein antibody 97/8,[23] 1:1,000) were reduced by ~50% ($p \leq 0.05$; Figure S6). Similarly, in P19 mouse neuroblastoma cells, immunoblot analysis demonstrated that shRNA-mediated knockdown of *Rab39b* resulted in ~50% reduction in α-synuclein steady-state levels ($p \leq 0.005$; Figure S6). Although the mechanism remains to be fully defined, these results suggest that downregulation of RAB39B results in dysregulation of α-synuclein homeostasis. We sequenced *RAB39B* in a cohort of 187 individuals with early-onset PD; they had been previously sequenced and shown not to have mutations in known PD-associated genes, including *SNCA* (MIM 163890), *PARK2* (MIM 602544), *DJ1* (MIM 602533), *PINK1* (MIM 608309), and *LRRK2* (MIM 609007).[24] This analysis did not identify any additional variants, suggesting that mutations in *RAB39B* are not a common cause of early-onset PD.

To determine whether the parkinsonism observed in the affected individuals resulted from PD, we investigated the neuropathology associated with the loss of *RAB39B*. Individual II:1 died at age 48 years from positional asphyxia. Postmortem neuropathological studies on the brain of II:1 were consistent with PD. The macroscopic findings were unremarkable, and serial coronal sections showed normal cortex, white matter, and ventricles with normal-appearing basal ganglia and thalamus. Cross section of the brain stem showed pallor of the SN and locus coeruleus. SN sections stained with haematoxylin and eosin (H&E) revealed hallmark neuropathological PD features, including loss of pigmented neurons and Lewy bodies in surviving neurons (Figure 3). Immunoreactive staining revealed the presence of α-synuclein-positive Lewy bodies and Lewy neurites in >10% of the surviving neurons. Additional neuropathological features included an abundance of cortical Lewy bodies, which are a pathological feature characteristic of dementia with Lewy bodies (DLB [MIM 127750]; reviewed in[27,28]). Tau-immunoreactive NFTs were also observed in a small proportion of the surviving pigmented SN neurons (Figure 3). Tau pathology has previously been observed in familial and idiopathic PD, and the tau-encoding gene (microtubule-associated protein tau [*MAPT* (MIM 157140)]) exists within a PD susceptibility locus. Tau plays a role in iron homeostasis,[29–31] and Perl staining revealing a modest accumulation of iron in the

SN (Figure 3) was consistent with the slight reduction in T2 signal intensity observed in individual II:1 (Table S1). In addition, analysis of the basal ganglia identified rare axonal spheroids in the white-matter tracts (data not shown), similar to the Wallerian-like degeneration observed in neurodegenerative diseases with impaired axonal transport.[32] The additional pathological and clinical features share similarities with other neurodegenerative disorders, the most similar of which was neurodegeneration with brain iron accumulation (NBIA [MIM 234200]). Notably, in rare cases, NBIA can manifest with developmental delay and subsequent early-onset parkinsonism.[33] Although MRI of II:3 was normal and II:1 did not show symptoms typical of NBIA,[34] genomic DNA from individuals with NBIA was analyzed. Ethics approval was provided by the IRB at Oregon Health & Science University, and informed consent was obtained. Sequence analysis of *RAB39B* in a cohort of 48 male individuals with NBIA of unknown etiology did not identify any variants.

In conclusion, genetic studies have demonstrated that loss of RAB39B causes pathologically defined PD, and functional studies have provided additional evidence for pathogenicity. Our results link the loss of a single gene involved in neuronal organization and synaptic function to the early manifestation of both ID and neurodegeneration and suggest that the loss of RAB39B dysregulates α-synuclein. For the two families we ascertained and clinically characterized, there appears to be a canonical age-dependent progression, namely ID first and then a slowly progressive basal ganglia disorder that advances after puberty. This was observed or reported in all 16 affected males in the Australian and Wisconsin families. However, given the lack of clinical data for the other two families previously described to be affected by *RAB39B* mutations,[17] it is unclear whether this age-dependent phenotype predominates or whether heterogeneous phenotypes extending from ID to ID with parkinsonism are associated with loss of RAB39B function. This issue will be resolved by future studies of additional individuals with mutations in *RAB39B*.

The proposed role of RAB39B in vesicular trafficking identifies a potential disease mechanism that is distinct from pathways associated with genes in which mutations are currently known to cause familial early-onset PD. Previous in vitro studies have demonstrated that α-synuclein-mediated deficits in vesicular trafficking can be ameliorated by the overexpression of several RAB proteins[35,36] but have not shown that loss of a specific RAB can cause PD. Current studies are further investigating how loss of RAB39B might cause the observed in vitro deficits in localization and reduced steady-state levels of α-synuclein but in vivo accumulation of significant α-synuclein pathology at end-stage disease. It is possible that in simple cell models with efficient protein-metabolism pathways, the "mislocalized" α-synuclein is rapidly turned over and thus leads to reduced steady-state levels. However, protein-turnover pathways are compromised in individuals with PD;[37] therefore, mislocalized α-synuclein might not be turned over efficiently, and as the disease progresses, the protein could accumulate and be incorporated into the protein aggregates that define PD.

The broader pathology of iron accumulation, NFTs, and axonal spheroids is similar to that reported for a range of neurodegenerative conditions. However, the abundance of both brainstem and cortical Lewy bodies suggests that RAB39B and/or associated pathways might directly contribute to the pathogenic mechanisms underlying dementia disorders such as DLB. Further studies, including the development of animal models, will be important for understanding the underlying pathogenic mechanism(s) of RAB39B dysfunction and identifying potential targeted therapeutic interventions.

Supplemental Data

Supplemental Data include six figures and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2014.10.015.

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Web Resources

The URLs for data presented herein are as follows:

ClinVar, http://www.ncbi.nlm.nih.gov/clinvar/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org/
PyMOL, http://www.pymol.org/
RefSeq, http://www.ncbi.nlm.nih.gov/refseq/

Accession Numbers

The ClinVar accession numbers for the *RAB39B* variants reported in this paper are SCV000190018 and SCV000190929.

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