J Mol Neurosci (2007) 33:151–154
DOI 10.1007/s12031-007-0030-7

Analysis of Candidate Genes at the IBGC1 Locus Associated with Idiopathic Basal Ganglia Calcification ("Fahr's Disease")

J. R. M. Oliveira · M. J. Sobrido · E. Spiteri · S. Hopfer · G. Meroni · E. Petek · M. Baquero · D. H. Geschwind

Received: 5 March 2007 / Accepted: 9 March 2007 / Published online: 1 August 2007

Abstract Basal ganglia calcification (striatopallidodentate calcifications) can be caused by several systemic and neurological disorders. Familial Idiopathic Basal Ganglia Calcification (IBGC, "Fahr's disease"), is characterized by basal ganglia and extrabasal ganglia calcifications, parkinsonism and neuropsychiatric symptoms. Because of an increased use of neuroimaging procedures, calcifications of the basal ganglia are visualized more often and precociously. In 1999, a major American family with IBGC was linked to a locus on chromosome 14q (IBGC1). Another small kindred, from Spain, has also been reported as possibly linked to this locus. Here we report the main findings of the first 30 candidate genes sequenced at the IBGC1 locus during the process of searching for a mutation responsible for familial IBGC. During the sequencing process, we identified a heterozygous nonsynonymous single nucleotide polymorphism (exon 20 of the MGEA6/c-TAGE gene) shared by the affected and not present in the controls. This SNP was randomly screened in the general population (348 chromosomes) in a minor allele frequency to 0.0058 (two heterozygous among 174 subjects). Another variation in this gene, in the exon 9, was found in the Spanish family. However, this variation was extremely common in the general population. Functional and population studies are necessary to fully access the implications of the MGEA6 gene in familial IBGC, and a complete sequencing of the IBGC1 locus will be necessary to define a gene responsible for familial IBGC.

Keywords Fahr's disease · Basal ganglia calcification · Parkinsonism · Sequencing · Neuropsychiatric disorders

J. R. M. Oliveira · M. J. Sobrido · E. Spiteri · S. Hopfer · D. H. Geschwind (✉)
The Neurogenetics Program and Department of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA90095-1769, USA
e-mail: dhg@ucla.edu

J. R. M. Oliveira
Neuropsychiatry Department & Keizo Asami Laboratory, Federal University of Pernambuco, Recife-PE, Brazil

G. Meroni
TIGEM c/o Area della Ricerca del CNR, Via Pietro, Castellino 111, 80131 Naples, Italy

E. Petek
Institute of Medical Biology and Human Genetics, Medical University of Graz, Graz 8010, Austria

M. Baquero
University Hospital La Fe, Valencia, Spain

Introduction

A number of systemic and neurological disorders can lead to basal ganglia calcifications (Sobrido and Geschwind 2002; Morita et al. 1998; Manyam 2005). Because of an increased use of neuroimaging procedures, calcifications of the basal ganglia are visualized more often and precociously (Schmidt et al. 2005; Shakibai et al. 2005). "Fahr type" calcification (striatopallidodentate calcifications) is a relatively common finding affecting 1–2% of patients undergoing diagnostic neuroimaging (Fujita et al. 2003).

Familial Idiopathic Basal Ganglia Calcification (IBGC or "Fahr's disease") is an inherited neurological condition characterized by basal ganglia and extrabasal ganglia calcifications, parkinsonism, dystonia, ataxia, and neuropsychiatric symptoms (Sobrido and Geschwind 2002).

Calcifications usually affect globus pallidus, putamen, caudate, and often also involve the thalamus, cerebellum, and subcortical white matter (Sobrido et al. 2002).

Most of these families display an autosomal dominant pattern of inheritance, but the etiology remains unknown. The first locus associated with IBGC (IBGC1) was found on the long arm of the chromosome 14 in a large multigenerational family (FY1; Geschwind et al. 1999). Another small kindred, from Spain, has also been reported as possibly linked to this locus, narrowing the candidate region to 10.9 cM (Oliveira et al. 2004).

Other families with IBGC have been excluded from the chromosome 14 region, indicating the possibility of genetic heterogeneity. An Australian pedigree was recently excluded from the IBGC1 locus (Brodaty et al. 2002) and it included 10 subjects with basal ganglia calcification, two of which had symptoms of dementia, parkinsonism, and mood disorder. We have also excluded this locus in families from China, Canada, and Germany (Sobrido and Geschwind 2002; Sobrido et al. 2002; Oliveira et al. 2004).

Another family with familial IBGC, in which linkage was not reported, presented pathological analysis revealing α-synuclein deposits in oligodentrocytes in the putamen, midbrain, and pons (Lhatoo et al. 2003). There appeared to be a pattern of anticipation, similar to the first family linked to the IBGC1 locus (Geschwind et al. 1999), with cases affected with the first symptoms ranging from 54 years old to possibly 10 years of age.

To find a mutation responsible for this phenotype, we performed sequencing of 26 candidate genes in the IBGC1 locus responsible for familial IBGC.

Methods

Subject Recruitment and Assessment

Two IBGC pedigrees were ascertained in a program approved by the UCLA Institutional Review Board, and informed consent was obtained. A brain computerized tomography (CT) scan was obtained to document the presence or absence of calcifications and define affection status. Biochemical investigation was performed in at least one affected in each family to rule out abnormalities of calcium regulation and metabolic disorders such as pseudohypoparathyroidism that could underlie brain calcifications (Geschwind et al. 1999). The first family(FY1) linked to the 14q has been previously reported (Geschwind et al. 1999). A second family (FS4) was linked to this same region (Oliveira et al. 2004) and it was used to confirm candidate single nucleotide polymorphisms (SNPs) founded in the FY1 family.

The most conservative criteria defining affectation was used. Defining affected is complicated by the heterogeneity in clinical presentation, age-dependent penetrance, and the fact that many asymptomatic individuals have positive CTs. Thus, we defined affecteds as those with positive CTs as we have previously described (Sobrido and Geschwind 2002; Sobrido et al. 2002; Oliveira et al. 2004). Those with negative CTs who are over the age of 50 are defined as unaffected, whereas those at earlier ages are classified as unknown because of the age-dependent penetrance for calcium deposits.

Selection of Candidate Genes

The candidate genes were chosen according to positional and/or functional criteria. “Positional candidates” are those genes that are located in regions of possible recombinations, to help narrow the candidates regions, or at the regions of higher multipoint lod scores. “Functional candidates” are those genes involved in metabolic pathways that might be relevant for the formation of calcium deposits or previously associated with brain calcifications. We performed sequencing of the coding region of the genes *EGLN3*, *SNX6*, *MBIP*, *TITF-1*, *NKX2.8*, *SLC25A21*, *PSMC6*, *SEC23A*, *HNF3A*, *TULIP 1*, *MGEA6*, *AKAP6*, *PSMA6*, *PAX9*, *SSTR1*, *SIP1*, *SOS2*, *ATPW*, *NIN*, *PYGL*, *TRIM9*, *PTGDR*, *STYX*, *BMP4*, *GCH1*, *HSPA2*.

Sample Collection and Candidate Gene Analysis

Informed consent was obtained and DNA was extracted from peripheral blood lymphocytes using the Puregene kit (Gentra Systems).

According to the annotation of coding sequence parts, primers were designed manually or by Primer 3 program (Rozen and Skaletsky 2000).

Polymerase chain reaction (PCR) fragments contained at least 50 bases of the 5′ and 3′ flanking regions for each exon. PCR was performed in 15 μl reaction volume containing 20 ng of subject DNA and) in a reaction mixture containing 2 μM $MgCl_2$, 200 μM dNTPs, 0.75 U *taq* DNA polymerase (Qiagen), 1× Qiagen PCR buffer and 0.2 μM of each primer under the following cycling conditions: 95°C for 5 min, then 30 cycles of 95°C for 30 s with annealing temperature optimized per primer pair, for 30 s and 72°C for 45 s, followed by a final extension of 72°C for 5 min. The PCR products were purified in 96 well plates with Sephadex G50 columns (Sigma, St Louise, MO, USA). All sequencing was performed by cycle sequencing and analyzed on an ABI 3700 Capillary DNA Analyzer (Perkin-Elmer, Foster, CA, USA). Traces were analyzed with SeqMan (DNASTAR Inc, https: www.dnastar.com/web/index.php) and BLAST to compare the sequences of affected subjects with controls

and the sequences available from the NCBI database (NCBI, http://ncbi.nlm.nih.gov/BLAST).

Results

Candidate Gene Analysis

We have sequenced the coding region of 26 genes, which comprises around one third of the known genes in the candidate region, including more than 300 exons (see Table 1). We found several novel SNPs. Most of the SNPs identified were also found in controls and/or caused synonymous changes. During this process, we characterized a new gene, *TULIP1*, an important candidate for neuropsychiatric conditions linked to the 14q13 region, but also excluded as a candidate for the IBGC1 locus (Schwarzbraun et al. 2004).

We identified a missense SNP at the exon 20 of the *MGEA6* gene (a transversion), coding for a coiled-coil proline-rich peptide (Comtesse et al. 2001). This was the first non-synonymous SNP (changing a proline per alanin aminoacid), found in all affected patients of the 14q-linked family and was absent in unaffected subjects from the family. The second family possibly linked to the IBGC1 locus (Oliveira et al. 2004) was also screened for mutations at this same gene and a novel SNP was also found, but at the exon 9.

Population Screening of the MGEA6 Variations

The exon 20 SNP changes a conserved Proline in a rich proline region and might have functional implications. We screened 348 chromosomes and found two heterozygous among samples from our lab control DNA bank and the Corriel cell repository, bringing the minor allelic frequency (MAF) of this SNP to 0.0058.

The exon 9 SNP was common and presenting MAF of 0.13.

Discussion

During the sequencing process, several novel SNPs have been identified and other predicted SNPs have been confirmed (see Table 1). Two nonsynonymous SNPs at the exon 20 and exon 9 of the *MGEA6* gene were shared by the affected and not presented in the controls.

The exon 20 SNP should be considered a rare variation based on the study of Freudenberg-Hua et al. (2003) who analyzed 65 candidate genes for Central Nervous System disorders and concluded that rare SNPs have MEF<0.05. The exon 9 SNP should be considered common (5.0%> MEF<20.0%).

Interestingly, the exon 20 of the MGEA6 genes is commonly spliced, generating the isoform MGEA 11, also expressed in the brain (Usener et al. 2003).

MGEA6 is a coil-coiled protein with a proline-rich region, expressed in several tissues including brain, highly expressed in meningioma, the most common benign brain tumor often presenting calcification visible at neuroimaging studies, especially CTs (Comtesse et al. 2001, 2002; Usener et al. 2003).

This type of proline-rich "signature" is predicted to be involved in protein–protein interaction, signal transduction,

Table 1 Sequence variants identified in chromosome 14 candidate genes

Gene	Location	Base change	Sequence	aa change	NCBI SNP database
TULIP 1	Intron 3 (exon 4–14)	A>C Het	CTTT A/C TATT		
	Intron 6 (exon 5 +100)	C>T Homo	ATCT T/C TGAA		
MGEA6	Exon 20@ +18	C>G Het	TGGT C/G CCTC	Pro521Ala	
	Exon 2@ + 331	C>T Homo	GGGG C/T TACC	Ala6Val	rs7140561
	Exon 9@ + 48	G>C Homo and Het	TGAA G/C ATAG	Lys205Asp	
	Intron 8 (exon 9 –122)	C>G Homo and Het	CTGC C/G TCTG		
AKAP6	Exon 15@	A>C	AGGG A/C AAAC	Gly1908Gly	rs2239647
	Exon 15@	A>G	ATGC A/G CTGA	Ala2001Ala	rs1051694
	Exon 15@	T>A	TGTT T/A CTCT	Phe2171Tyr	rs4647899
PSMA6 intronic repeats *	Intron 5 (exon 6 +491)	TG_{21}=*control and affecteds*	TGGA TG_n TTCT		
PAX9	Exon 4@ +86	C>T	CGCA C/T GCGG	His239His	
	Exon 4@ +87	G>C	GCAC G/C CGGT	Ala240Pro	rs4904210
SSTR1	Exon 1@	T>C	TGGT T/C AACG	Val293Val	rs2228497
SIP1	UTR	C>T	GGCG C/T ACTA		rs2277458
TRIM9	Exon 10@	A>C Het	ACTT A/C AATA	Leu 653Phe	rs2275462
	Intron 6 (exon 6 +4)	T>A Homo and Het	GGTA T/A GTCC		rs2297889
	Intron 2 (exon 2 +27)	A>G	AAGG A/G AACC		

and signaling pathways with WW (tryptophan rich) and SH3 domains. The signaling complexes they mediate have been implicated in several human diseases including muscular dystrophy, Huntington's disease and Alzheimer's disease (Sudol et al. 2001).

Proline residues play an important role in the structure and function of various proteins. The insertion of an 217 alanin (a nonpolar side-chain aa), because of the mutation, 218 would definitely have important implications to the tri- 219 dimensional structure of this protein. Because of lack of an 220 amide proton, proline residues are not hydrogen bond 221 donors. As a result of these properties, prolines often induce 222 helix bending or are part of tight turns in three-dimensional 223 (3D) structures of proteins (Sansom and Weinstein 2000; 224 Macias et al. 1996, 2002).

No conclusion regarding pathogenicity should be drawn, but considering the growing body of evidence suggesting that basal ganglia calcification is a relatively common finding, functional and population studies are necessary to fully access the implications of the *MGEA6* gene in familial IBGC and a complete sequencing of the IBGC1 locus will be necessary to define a gene responsible for familial IBGC. Additional studies are in progress to analyze the impact of this SNP in the expression of this protein and its repercussions in CNS and eventually in familial IBGC.

References

Brodaty, H., Mitchell, P., Luscombe, G., Kwok, J. J., Badenhop, R. F., McKenzie, R., et al. (2002). Familial idiopathic basal ganglia calcification (Fahr's disease) without neurological, cognitive and psychiatric symptoms is not linked to the IBGC1 locus on chromosome 14q. *Human Genetics*, *110*, 8–14.

Comtesse, N., Niedermayer, I., Glass, B., Heckel, D., Maldener, E., Nastainczyk, W., et al. (2002). MGEA6 is tumor-specific over-expressed and frequently recognized by patient-serum antibodies. *Oncogene*, *21*, 239–247.

Comtesse, N., Reus, K., & Meese, E. (2001). The MGEA6 Multigene family has the active locus on 14q and least nine psudogenes on different chromosomes. *Genomics*, *75*(1–3), 1–6.

Freudenberg-Hua, Y., Freudenberg, J., Kluck, N., Cichon, S., Propping, P., & Nothen, M. M. (2003). Single nucleotide variation analysis in 65 candidate genes for CNS disorders in a representative sample of the European population. *Genome Research*, *13*(10), 2271–2276.

Fujita, D., Terada, S., Ishizu, H., Yokota, O., Nakashima, H., Ishihara, T., et al. (2003). Immunohistochemical examination on intracranial calcification in neurodegenerative diseases. *Acta Neuropathologica*, *105*, 259–264.

Geschwind, D. H., Loginov, M., & Stern, J. M. (1999). Identification of a locus on chromosome 14q for idiopathic basal ganglia calcification (Fahr disease). *American Journal of Human Genetics*, *65*, 764–772.

Lhatoo, S. D., Perunovic, B., Love, S., Houlden, H., & Campbell, M. J. (2003). Familial idiopathic brain calcification—A new and familial alpha-synucleinopathy? *European Neurology*, *49*, 223–226.

Macias, M. J., Hyvonen, M., Baraldi, E., Schultz, J., Sudol, M., Saraste, M., et al. (1996). Structure of the WW domain of a kinase-associated protein complexed with a proline-rich peptide. *Nature*, *382*, 646–649.

Macias, M. J., Wiesner, S., & Sudol, M. (2002). WW and SH3 domains, two different scaffolds to recognize proline-rich ligands. *FEBS Letters*, *513*, 30–37.

Manyam, B. V. (2005). What is and what is not 'Fahr's disease'. *Parkinsonism & Related Disorders*, *11*(2), 73–80.

Morita, M., Tsuge, I., Matsuoka, H., Ito, Y., Itosu, T., Yamamoto, M., et al. (1998). Calcification in the basal ganglia with chronic active Epstein–Barr virus infection. *Neurology*, *50*, 1485–1488.

Oliveira, J., Sobrido, M. J., Hopfer, S., Spiteri, E., Klepper, J., Gilbert, J., et al. (2004). Genetic heterogeneity in Familial Idiopathic basal Ganglia Calcification ("Fahr's disease"). *Neurology*, *63*, 2165–2167.

Rozen, S., & Skaletsky, H. (2000). Primer3 on the WWW for general users and for biologist programmers. *Methods in Molecular Biology*, *132*, 365–386.

Sansom, M. S. P., & Weinstein, H. (2000). Hinges, swivels and switches: the role of pralines in signaling via transmembrane α-helices. *TiPS*, *21*, 445–451.

Schmidt, U., Mursch, K., & Halatsch, M. E. (2005). Symmetrical intracerebral and intracerebellar calcification ("Fahr's disease"). *Functional Neurology*, *20*(1), 15.

Schwarzbraun, T., Vincet, J. B., Schumacher, A., Geschwind, D. H., Oliveira, J., Windpassinger, C., et al. (2004). Cloning of TULIP1, a novel CpG island associated, brain expressed candidate gene for 14q13-linked neurological phenotypes and its murine homologue. *Genomics*, *84*(3), 577–586.

Shakibai, S. V., Johnson, J. P., & Bourgeois, J. A. (2005). Paranoid delusions and cognitive impairment suggesting Fahr's disease. *Psychosomatics*, *46*(6), 569–572.

Sobrido, M. J., & Geschwind, D. H. (2002). Genetics of familial idiopathic basal ganglia calcification (FIBGC). In S.-M. Pulst (Ed.), *Genetics of movement disorders* (pp. 443–448). San Diego: Academic Press.

Sobrido, M. J., Hopfer, S., & Geschwind, D. H. (2002). *Idiopathic basal ganglia calcification*. GeneReviews: Genetic Disease Online Reviews. www.geneclinics.org.

Sudol, M., Krzystof, S., & Russo, T. (2001). Functions of WW domains in the nucleus. *FEBS Letters*, *490*, 190–195.

Usener, D., Schadendorf, D., Kock, J., Dubel, S., & Eichmuller, S. (2003). cTAGE: A cutaneous T Cell Lymphoma associated antigen family with tumor specific splicing. *The Journal of Investigative Dermatology*, *121*, 198–206.