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Sequence analysis of P21-activated kinase 3 (PAK3) in chronic schizophrenia with cognitive impairment

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ABSTRACT

The P21-activated kinase PAK3 is critical for cognitive development and truncating mutations cause non-syndromic mental retardation (MR). Missense mutations are also associated with psychotic disorders, most commonly with schizophrenia involving premorbid MR, namely "pfropfschizophrenie". We set out to measure the frequency of sequence variants in *PAK3* in schizophrenia without premorbid MR. We conducted complete gene reseqeuncing of all coding exons and exon–intron boundaries in patients with schizophrenia with cognitive impairment but without premorbid MR. Deleterious variants in schizophrenia alone were rare (<1/159 or 0.6%). Thereby, while PAK3 remains a strong biological candidate in psychosis, evidence from human genetics provides strongest support for a link to pfropfschizophrenie and not to schizophrenia without premorbid intellectual disability.

1. Introduction

Abnormalities in GABAergic interneurons (Lewis et al., 2005) and loss of dendritic spines (Kolluri et al., 2005; Rosoklija et al., 2000) are consistent cellular abnormalities in schizophrenia. The *p*21 (CDKN1A)-*a*ctivated *k*inase PAK3 is involved in both interneuron development (Cobos et al., 2007) as well as actin remodeling in dendrites (Hayashi et al., 2004; Kreis et al., 2007). In addition to being a plausible biological candidate in schizophrenia susceptibility, there are strong human genetic data that support a role for the X-linked *PAK3* gene in psychotic disorders. In particular, multiple families with *PAK3* mutations have members with psychosis and premorbid mild mental retardation (Gedeon et al., 2003; Peippo et al., 2007; Rejeb et al., in press), known as "pfropfschizophrenie" (defined in Doody et al., 1998). Indeed, we contend that while *DISC1* has received a great deal of attention based largely on the discovery of a highly penetrant translocation in a single extended pedigree (St Clair et al., 1990), highly penetrant mutations in *PAK3* in psychosis have been largely overlooked. *PAK3* is critical for cognitive development and truncating mutations cause moderate, non-syndromic mental retardation (MR) (Allen et al., 1998); however, missense mutations are associated with neurobehavioral disorders (and milder MR) in all pedigrees described (Donnelly et al., 1996; Gedeon et al., 2003; Peippo et al., 2007; Rejeb et al., in press). Three of four such families have associated psychotic illness (Gedeon et al., 2003; Peippo et al., 2007; Rejeb et al., in press). Indeed, in an extended pedigree with an A365E mutation, mental retardation was generally mild and at least two members had psychotic illness, one with chronic paranoid schizophrenia and an IQ of 80 (Gedeon et al., 2003).

In this experiment, we set out to measure the frequency of sequence variants in *PAK3* in patients ascertained for schizophrenia without premorbid intellectual disability. We specifically chose a schizophrenia patient population with profound cognitive impairment with the notion that this population may be relatively enriched for *PAK3* mutations.

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2. Materials and methods

2.1. Patient ascertainment

Study procedures were approved by the Partners HealthCare and Massachusetts Department of Mental Health institutional review boards. After providing written informed consent, 159 outpatients with schizophrenia (43 females and 116 males) who receive treatment at an urban community mental health center in Boston were enrolled consecutively. The diagnosis of schizophrenia was confirmed by a research psychiatrist using DSM-IV criteria. Inclusion criteria included DSM-IV diagnosis of schizophrenia, age 18–70, ability to complete symptom rating scales and cognitive tests. Exclusion criteria included psychosis secondary to a substance use disorder or a general medical condition. Of enrolled subjects, 120 were Caucasian (75%), 37 were of African descent (23%), one was of East/Southeast Asian descent (1%), and one was of Latino descent (1%). All patients were medicated and stable in terms of psychotic symptoms.

2.2. Cognitive assessment

The Wechsler Adult Intelligence Scale (WAIS-III) was also administered to assess global cognitive status. Subnormal IQ was generally construed to be due to cognitive abnormalities of schizophrenia; however, psychotic symptoms and medication effects cannot be excluded as contributory factors.

2.3. Sequence analysis

All coding exons and exon–intron boundaries in *PAK3* were sequenced. Each amplicon was amplified by PCR and sequenced using capillary methods. Sequence reads were made first manually and secondarily by the *SNP-Detector* software package.

Table 1

Exon	Amplicon (hg18 genomic assembly)	Sequence variation
1	chrX:110,252,938–110,253,212	No variation
2	chrX:110,271,930–110,272,130	No variation
3	chrX:110,276,355–110,276,499	No variation
4	chrX:110,277,571–110,277,824	No variation
5	chrX:110,282,244–110,282,381	rs16986385 (18 males, 10 females — heterozygous), intronic SNP, 25 bp from SA site
6	chrX:110,292,749–110,292,980	No variation
7	chrX:110,293,396–110,293,661	No variation
8	chrX:110,302,852–110,303,015	No variation
9	chrX:110,321,961–110,322,109	No variation
10	chrX:110,322,340–110,322,552	No variation
11	chrX:110,324,135–110,324,352	No variation
12	chrX:110,325,676–110,325,875	chrX:110,325,800, C-> T, Leu395->Leu395 (1 female — heterozygous), novel SNP
13	chrX:110,326,278–110,326,574	No variation
14	chrX:110,346,255–110,346,492	rs10521535 (6 males, 6 females — heterozygous), intronic SNP, 40 bp from the SD site
15	chrX:110,350,192–110,350,381	No variation

3. Results

In female subjects, IQ ranged from 66 to 137 with six females under IQ of 70 and an overall mean female IQ of 86.3 +/− 16.7. In male subjects, IQ ranged from 59 to 130 with eight males under IQ of 70 and mean male IQ of 89.1 +/− 14.0. While no patients were known to have premorbid MR, cognitive status was significantly impaired and frequently in the mild to borderline MR range. Coding sequence across *PAK3* is known to be highly invariant (http://www.ncbi.nlm.nih.gov/projects/SNP/). There are a total of 242 known SNPs across the 124,561 bp genomic interval and all but 1 outside the 1635 open reading frame. One coding non-synonymous SNP (rs1805077) has been reported (but not validated), A489C, which putatively changes Arginine 31 to Serine. On re-sequencing in the schizophrenia patient sample, three total SNPs were discovered. We did not find rs1805077. Two of the SNPs had been previously discovered and were in intronic regions, namely rs16986385 and rs10521535 (Table 1). The single novel SNP discovered in the patient sample occurred in exon 12, substituting C at mRNA position 1786 for a T, and resulting in a synonymous SNP at Leucine 395. This novel SNP was found in a Caucasian female patient with normal IQ who was heterozygous for this sequence change.

4. Discussion

While four mutations in *PAK3* have been reported to cause MR and also to have neurobehavioral disorders in affected members of the pedigree (Donnelly et al., 1996; Gedeon et al., 2003; Peippo et al., 2007; Rejeb et al., in press), including three with psychotic illness (Gedeon et al., 2003; Peippo et al., 2007; Rejeb et al., in press), the frequency of potentially causative rare sequence variants in *PAK3* is less than one in 159 cases of schizophrenia alone (less than 0.6%). Variants do not appear to be enriched by selecting for patients with impaired cognitive function. Kraeplin named a schizophrenia subtype in 1907 “pfropfschizophrenie” for schizophrenia engrafted on top of intellectual disability (Doody et al., 1998). Each pedigree thus far reported with *PAK3* mutations has been ascertained for mild to moderate mental retardation as a primary diagnosis. The risk of psychotic illness is elevated in the presence of premorbid intellectual disability (Zammit et al., 2004) but generally quoted as less than 8% (Reid, 1980). As with mental retardation, in schizophrenia, cognitive impairment is a core feature and neurodevelopment abnormalities are prevalent. *PAK3* mutations may represent a genetic susceptibility for pfropfschizophrenie and an important shared biological component between MR and schizophrenia, although our data here indicate that sequence mutations or variants are rare in schizophrenia without premorbid MR. Additional experiments are now needed to define the frequency of *PAK3* sequence variants in schizophrenia with premorbid intellectual disability.

Role of funding source

The funding sources had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

D.C.G. was involved in recruitment of patient subjects. E.M.M., A.K. and C. A.W. were involved in preparation of DNA, gene sequencing and data

analysis. All were involved in hypothesis generation and preparation of this manuscript.

Conflict of interest

E.M.M. has received grant funding from Pfizer and Merck. Dr. Goff has received research funding or honoraria from, or has been on an advisory board of AstraZeneca, BMS, Cephalon, Cortex, Eli Lilly, GSK, Janssen, Merck, Organon, Pfizer, Solvay and Wyeth. All other authors declare that they have no conflicts of interest.

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References

Allen, K.M., Gleeson, J.G., Bagrodia, S., Partington, M.W., MacMillan, J.C., Cerione, R.A., Mulley, J.C., Walsh, C.A., 1998. PAK3 mutation in nonsyndromic X-linked mental retardation. Nat. Genet. 20 (1), 25–30.

Cobos, I., Borello, U., Rubenstein, J.L., 2007. Dlx transcription factors promote migration through repression of axon and dendrite growth. Neuron 54 (6), 873–888.

Donnelly, A.J., Partington, M.W., Ryan, A.K., Mulley, J.C., 1996. Regional localisation of two non-specific X-linked mental retardation genes (MRX30 and MRX31). Am. J. Med. Genet. 64 (1), 113–120.

Doody, G.A., Johnstone, E.C., Sanderson, T.L., Owens, D.G., Muir, W.J., 1998. 'Pfropfschizophrenie' revisited. Schizophrenia in people with mild learning disability. Br. J. Psychiatry 173, 145–153.

Gedeon, A.K., Nelson, J., Gecz, J., Mulley, J.C., 2003. X-linked mild non-syndromic mental retardation with neuropsychiatric problems and the missense mutation A365E in PAK3. Am. J. Med. Genet. A. 120 (4), 509–517.

Hayashi, M.L., Choi, S.Y., Rao, B.S., Jung, H.Y., Lee, H.K., Zhang, D., Chattarji, S., Kirkwood, A., Tonegawa, S., 2004. Altered cortical synaptic morphology and impaired memory consolidation in forebrain- specific dominant-negative PAK transgenic mice. Neuron 42 (5), 773–787.

Kolluri, N., Sun, Z., Sampson, A.R., Lewis, D.A., 2005. Lamina-specific reductions in dendritic spine density in the prefrontal cortex of subjects with schizophrenia. Am. J. Psychiatry 162 (6), 1200–1202.

Kreis, P., Thevenot, E., Rousseau, V., Boda, B., Muller, D., Barnier, J.V., 2007. The p21- activated kinase 3 implicated in mental retardation regulates spine morphogenesis through a Cdc42-dependent pathway. J. Biol. Chem. 282 (29), 21497–21506.

Lewis, D.A., Hashimoto, T., Volk, D.W., 2005. Cortical inhibitory neurons and schizophrenia. Nat. Rev., Neurosci. 6 (4), 312–324.

Peippo, M., Koivisto, A.M., Sarkamo, T., Sipponen, M., von Koskull, H., Ylisaukko-oja, T., Rehnstrom, K., Froyen, G., Ignatius, J., Jarvela, I., 2007. PAK3 related mental disability: further characterization of the phenotype. Am. J. Med. Genet. A. 143 (20), 2406–2416.

Reid, A.H., 1980. Psychiatric disorders in mentally handicapped children: a clinical and follow-up study. J. Ment. Defic. Res. 24 (Pt 4), 287–298.

Rejeb, I., Saillour, Y., Castelnau, L., Julien, C., Bienvenu, T., Taga, P., Chaabouni, H., Chelly, J., Jemaa, L.B., Bahi-Buisson, N., in press. A novel splice mutation in PAK3 gene underlying mental retardation with neuropsychiatric features. Eur. J. Hum. Genet. [Electronic publication ahead of print, June 4, 2008].

Rosoklija, G., Toomayan, G., Ellis, S.P., Keilp, J., Mann, J.J., Latov, N., Hays, A.P., Dwork, A.J., 2000. Structural abnormalities of subicular dendrites in subjects with schizophrenia and mood disorders: preliminary findings. Arch. Gen. Psychiatry 57 (4), 349–356.

St Clair, D., Blackwood, D., Muir, W., Carothers, A., Walker, M., Spowart, G., Gosden, C., Evans, H.J., 1990. Association within a family of a balanced autosomal translocation with major mental illness. Lancet 336 (8706), 13–16.

Zammit, S., Allebeck, P., David, A.S., Dalman, C., Hemmingsson, T., Lundberg, I., Lewis, G., 2004. A longitudinal study of premorbid IQ score and risk of developing schizophrenia, bipolar disorder, severe depression, and other nonaffective psychoses. Arch. Gen. Psychiatry 61 (4), 354–360.