A comprehensive screening of copy number variability in dementia with Lewy bodies

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Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

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https://doi.org/10.1016/j.neurobiolaging.2018.10.019
1. Introduction

Dementia with Lewy bodies (DLB) is a common and complex form of neurodegenerative disease, and its diagnosis can often be complicated by phenotypic similarities with Alzheimer’s disease (AD), Parkinson’s disease (PD), or even frontotemporal dementia (FTD) (Claassen et al., 2008; Heidebrink, 2002). A more accurate DLB diagnosis is usually obtained by integrating clinical and pathological data from brain autopsy (McKeith et al., 2017).

Genetic studies in DLB have been limited, certainly in comparison with studies on AD or PD, for a number of reasons, most notably because DLB has not been historically considered a genetic disease, given the lack of multiplex kindreds where the disease segregates. In addition, large cohorts of patients are difficult to collect given the frequency of the disease and the rate of misdiagnosis. Despite this, recent studies have conclusively shown that there is a role for genetics in the etiology of DLB (Bras et al., 2014; Guerreiro et al., 2018, 2016; Nalls et al., 2013; Peuralinna et al., 2015). Exome sequencing studies have been performed in small cohorts, as have case studies and Sanger sequencing of specific target genes (Clark et al., 2009; Geiger et al., 2016; Keogh et al., 2016; Koide et al., 2002; Ohtake et al., 2004). Copy number variation (CNV) has not been assessed thus far in DLB, particularly in an unbiased manner and at a genome-wide level.

CNVs have been widely studied in a number of neurological conditions, particularly in developmental phenotypes such as schizophrenia and autism (Glessner et al., 2009; Marshall et al., 2017; McCarthy et al., 2009) where several microdeletions and microduplications (100–600 kb) have been found to be associated with both diseases (Bassett et al., 2017; Cook et al., 1997; McCarthy et al., 2009; Stefansson et al., 2008; Weiss et al., 2008). In these phenotypes, CNVs play a prominent role in the disease genetic architecture.

Several studies have analyzed CNVs in AD, where APP duplications have been unequivocally shown to cause disease (Delabar et al., 1987; Ghani et al., 2012; Swaminathan et al., 2011, 2012; Zheng et al., 2014, 2015). In PD, pathogenic CNVs are also known to occur in SNCA, PARK2, PINK1, and PARK7 (Bonifati et al., 2003; Chartier-Harlin et al., 2004; Ibáñez et al., 2004; Lesage et al., 2008; Marongiu et al., 2007; Waters and Miller, 1994). Together, these data show that CNVs are an important mutational event in neurological conditions.

Here, we report the first genome-wide analysis of CNVs in DLB in a large cohort of patients, many of which with neuropathology diagnoses of DLB. We performed a case-control association study that was complemented by discovery stage analyses guided by candidate genes and CNVs previously reported as being associated with DLB-related neurodegenerative diseases.
information has been described previously (Guerreiro et al., 2018). Data from 1525 control samples were obtained from The Genetic Architecture of Smoking and Smoking Cessation study (phs000404.v1.p1) publicly available at the database of genotypes and phenotypes (https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000404.v1.p1). Supplementary Fig. 1 shows an overview of the study design, different quality control (QC) steps, and analyses performed.

2.2. Genotyping, quality control, and CNV calling

Seven hundred fifty-four DLB samples were genotyped on HumanOmni2.5Exome-8 v1.0B Illumina arrays, and 700 DLB samples were genotyped using Infinium OmniExpress-24 v1.2.A1 Illumina arrays (Illumina, Inc, CA, USA). Control samples were genotyped on HumanOmni2.5-4 v1.D arrays (Illumina, Inc, CA, USA). Intensity files were analyzed using GenomeStudio v2011.1 software (Illumina, Inc, CA, USA) along with the respective manufacturer's cluster files. QC procedures were performed in GenomeStudio (GS) before CNV analysis as described by Jarick et al. (Jarick et al., 2014). In short, samples with call rates lower than 0.97 were filtered out. SNP statistics were recalculated following visual inspection of B allele frequency (BAF) and log R ratio (LRR) plots. SNPs with GenTrain scores below 0.7 were excluded. Finally, samples with substantial cryptic relatedness scores (PHAT > 0.1) were removed, as previously described (Guerreiro et al., 2018).

CNV calls were generated using 2 different algorithms: cnvPartition v2.3.0 (Illumina, Inc) and PennCNV v1.0.4 (Wang et al., 2007). CNV calling based on cnvPartition was performed by GS with default parameters. For PennCNV, probe positions, LRR, and BAF values for samples that passed QC procedures were exported from GS. Population frequency of the B allele (PFB) file was calculated for each array separately. All smoking cessation samples were used to generate CNVs in cnvPartition and PennCNV, but only a subset of the best performing 700 samples was used for the compilation of the PFB file in PennCNV to match the number of samples used for cases. PennCNV GC-model files were then created based on these PFBs. Finally, CNVs were inferred by PennCNV using the hidden Markov model and the GC-model for wave adjustment. Calls for the X chromosome were generated separately. Chromosome Y SNPs were not analyzed.

2.3. CNV quality control and analysis

To improve the quality of CNVs, only calls generated by both algorithms were kept, whereas calls made by a single algorithm or calls of opposing type (e.g., assigned as a deletion by one algorithm and as duplication by the other) were discarded. Adjacent CNVs were merged if the length of the sequence between them was smaller than 50% of the length of the larger CNV (Mok et al., 2016). CNVs were excluded if they were overlapping telomeres, centromeres, known segmental duplications, the immunoglobulin, or T cell receptor loci. Samples having LRR SD > 0.28, BAF drift > 0.002, waviness factor > 0.04, or having more CNV calls than 3'sD + median were excluded (Marshall et al., 2017; Need et al., 2009).

To identify potentially pathogenic CNVs, we analyzed CNVs spanning known genes. We used the database of genomic variants (DGV) (http://dgv.tcag.ca/dgv/app/home, accessed November 2017) to determine the population frequency of CNVs (MacDonald et al., 2014). This information was complemented with the frequency from clinical samples available in DECIPHER v9.18 (https://decipher.sanger.ac.uk/, accessed November 2017).

2.4. Case-control association analysis

Case-control association analysis was implemented using ParseCNV (Glessner et al., 2013). Standard ParseCNV quality metrics were used to filter out low-quality results. CNVs that were genome-wide significant [p-value < 5 × 10^{-4} as suggested by (Glessner et al., 2013)] had a minimum length of 50 kb, and passed visual inspection in GS were selected for further analyses.

2.5. Candidate CNVs approach

CNVs previously described in AD (Ghani et al., 2012; Heinzen et al., 2010; Swaminathan et al., 2011, 2012; Zheng et al., 2014, 2015), PD (Bademci et al., 2010; Liu et al., 2013; Mok et al., 2016; Pankratz et al., 2011), and FTD (Gijselinck et al., 2008) were specifically investigated in these data (Supplementary Table 1). This analysis was performed on the complete set of CNV results after QC, disregarding the filters used for the case-control association analysis performed by ParseCNV.

2.6. Candidate genes approach

CNVs located in known AD, PD, FTD, and DLB genes were also assessed (Brás et al., 2015; Guerreiro et al., 2013, 2015, 2018; Jansen et al., 2015; Keogh et al., 2016; Koide et al., 2002; Ohtake et al., 2004; Saitoh et al., 1995). Supplementary Table 2 lists all genes studied using this approach.

3. Results

3.1. CNV calling and QC steps

After QC steps at the GS level, a total of 2819 samples (1294 cases and 1525 controls) remained for further analyses. From the 754 DLB samples genotyped with HumanOmni2.5 arrays, 616 (81.7%) samples were kept and from the 2,443,177 probes in this array, 97.8% of the 2,443,177 probes were kept.

Five CNVs previously associated with DLB-related neurodegenerative diseases were found in DLB patients (Table 1). Two of these
<table>
<thead>
<tr>
<th>Genes</th>
<th>Location</th>
<th>CNV</th>
<th>p-value</th>
<th>Cases frequency</th>
<th>Controls frequency</th>
<th>DCV frequency</th>
<th>DECIPHER frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Case-control association analysis</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>ADGRC7,TFG</td>
<td>chr3:100,357,671-100,439,759</td>
<td>Gain</td>
<td>$8.93 \times 10^{-5}$</td>
<td>21 (18)</td>
<td>13</td>
<td>$1.77 \times 10^{-2}$</td>
<td>$9.10 \times 10^{-3}$</td>
</tr>
<tr>
<td>PDZD2</td>
<td>chr5:32101,400-32,106,628</td>
<td>Gain</td>
<td>$2.94 \times 10^{-6}$</td>
<td>14 (12)</td>
<td>0</td>
<td>$1.18 \times 10^{-2}$</td>
<td>0</td>
</tr>
<tr>
<td>LAPT4M8</td>
<td>chr6:98795,434-98,800,334</td>
<td>Loss</td>
<td>$6.29 \times 10^{-7}$</td>
<td>12 (6)</td>
<td>0</td>
<td>$1.01 \times 10^{-2}$</td>
<td>0</td>
</tr>
<tr>
<td>MSR1</td>
<td>chr8:15948,235-16,021,468</td>
<td>Loss</td>
<td>$1.20 \times 10^{-4}$</td>
<td>13 (7)</td>
<td>4</td>
<td>$1.10 \times 10^{-2}$</td>
<td>$2.80 \times 10^{-3}$</td>
</tr>
<tr>
<td>NME1,NME1,NME2,SPAG9</td>
<td>chr17:49,177,096-49,231,786</td>
<td>Loss</td>
<td>$2.72 \times 10^{-4}$</td>
<td>9 (4)</td>
<td>0</td>
<td>$7.58 \times 10^{-3}$</td>
<td>0</td>
</tr>
<tr>
<td><strong>Candidate CNVs approach</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CSMD1</td>
<td>chr8:4,033,908-4,126,540</td>
<td>Loss</td>
<td>$2.89 \times 10^{-2b}$</td>
<td>3 (2)</td>
<td>0</td>
<td>$2.53 \times 10^{-3}$</td>
<td>0</td>
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<tr>
<td>DDX11,OVOS2</td>
<td>chr12:31,249,834-31,407,303</td>
<td>Gain</td>
<td>$1.41 \times 10^{-2b}$</td>
<td>4 (2)</td>
<td>0</td>
<td>$3.37 \times 10^{-3}$</td>
<td>0</td>
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<tr>
<td>CSMD1</td>
<td>chr16:29,595,483-29,912,902</td>
<td>Gain</td>
<td>na</td>
<td>5 (5)</td>
<td>8</td>
<td>$4.21 \times 10^{-3}$</td>
<td>$5.60 \times 10^{-3}$</td>
</tr>
<tr>
<td><strong>Candidate genes approach</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNAJC6,LEPR,LEPROT</td>
<td>chr1:65,854,556-65,955,725</td>
<td>Gain</td>
<td>na</td>
<td>1 (0)</td>
<td>1</td>
<td>$8.42 \times 10^{-4}$</td>
<td>0</td>
</tr>
<tr>
<td>SNCA,SNCA-AS1,CHR1,MRK1,CCSER1</td>
<td>chr4:90,035,549-91,420,358</td>
<td>Gain</td>
<td>na</td>
<td>1 (1)</td>
<td>0</td>
<td>$8.42 \times 10^{-4}$</td>
<td>0</td>
</tr>
<tr>
<td>PARK2</td>
<td>chr6:161,601,162-163,259,260</td>
<td>Loss or Gain</td>
<td>na</td>
<td>13 (8)</td>
<td>28</td>
<td>$1.10 \times 10^{-2}$</td>
<td>$1.96 \times 10^{-2}$</td>
</tr>
<tr>
<td>GABRB3,GABRA5,GABRG3</td>
<td>chr15:26,996,126-27,220,713</td>
<td>Gain</td>
<td>na</td>
<td>1 (1)</td>
<td>0</td>
<td>$8.42 \times 10^{-4}$</td>
<td>0</td>
</tr>
<tr>
<td>MAPT,CHR1,KANSL1,KANSL1-AS1,MAPT-AS1,MAPT-IT1,SPPL2C,STH</td>
<td>chr17:43,661,362-44,345,063</td>
<td>Gain</td>
<td>na</td>
<td>2 (0)</td>
<td>0</td>
<td>$1.68 \times 10^{-3}$</td>
<td>0</td>
</tr>
<tr>
<td>APPADAMTS1,ADAMTS5,ATP5J,CRYR1,CRYR1-AS1,GABPA,JAM2,MRP139</td>
<td>chr21:25,063,840-28,522,487</td>
<td>Gain</td>
<td>na</td>
<td>1 (0)</td>
<td>0</td>
<td>$8.42 \times 10^{-4}$</td>
<td>0</td>
</tr>
<tr>
<td>CHCHD10,ADORA2A,ADORA2A-AS1,CC2orf15,CABIN1,GSSAP1,DDX1,DDOT1,DELB3,DRCH1,LGFT1,GFT5,GSTT1,GSTT1-AS1,GSTT2,GSTT2B,GSTTP1,GSTTP2,GDC1,GUSBP11,ICL11,LRCC75B,MIF,MIF-AS1,MMP11,POM121L9P,NDUFA6-AS1,POM121L9P,PHD3,ODC11,ODH2,ODAD2,SPEC1L,SUSD2,UBP1,VPREB3, ZDHHC8P1,ZN70</td>
<td>chr22:23,690,325-25,011,417</td>
<td>Gain</td>
<td>na</td>
<td>1 (1)</td>
<td>0</td>
<td>$8.42 \times 10^{-4}$</td>
<td>0</td>
</tr>
</tbody>
</table>

All genomic coordinates are for the genome assembly hg19.

\textsuperscript{a} Neuropathological diagnosis; na—not applicable; p-value—these were calculated including all cases (not only neuropathologically confirmed cases).

\textsuperscript{b} p-values resulting from the case-control association analysis.
were present in the control group with a higher frequency than in the patient group, and the remaining 3 are described in public databases and are detailed next. The duplication identified on chromosome 12 overlapping DDX11 and OVO2 has a frequency 10-fold higher in DGV than in the DLB cohort. The 16p11.2 microduplication found in one DLB patient has a frequency of $1.69 \times 10^{-4}$ in the DECIPHER database but does not occur in any control samples or in the DGV database. One of the CNVs that was previously significantly associated with AD locates at chr8:2,792,874-4,852,328 and overlaps CSM1 (Swaminathan et al., 2011). CSM1 has frequent deletions and duplications spanning the whole gene as reported in DGV. Our results agree with this: at this locus, we identified over 100 deletions in cases and controls. One region in the gene has a deletion in 3 cases and in no controls and this CNV showed a suggestive significance in our DLB case-control association analysis. Therefore, we only report this shorter region in Table 1.

### 3.4. Candidate genes approach

We investigated CNVs in genes known to be associated with diseases that are related to DLB (Supplementary Table 2) and identified a total of 8 CNVs (Table 1). These included one duplication in APP occurring in a clinically diagnosed case. This large duplication is not present in the databases or in the control cohort. Two DLB subjects were found to carry duplications spanning MAPT, and one neuropathologically diagnosed patient was found to carry a SNCA duplication (Fig. 2). PARK2 was found to have many copy number losses and gains in controls ($n = 28$) and cases ($n = 13$) but none were homozygous. A duplication including CHCHD10 was also identified in a neuropathologically diagnosed DLB patient. No CNVs were identified in GBA, the gene presenting the strongest association with DLB.

### 4. Discussion

We performed a systematic analysis of CNVs in a large cohort of DLB patients using 3 main approaches. The first of these approaches was a case-control association analysis, which resulted in 5 significant CNV regions that have not been previously described as associated with the disease. The most significant result from this analysis was a deletion spanning the lysosome-associated transmembrane protein, LAPTMS. Intra-neuronal alpha-synuclein clearance likely occurs through a variety of mechanisms to maintain protein homeostasis. However, recent data have highlighted the importance of lysosomal pathways for degradation of this protein (Webb et al., 2003). Interestingly, a member of the same protein family, LAPTMS, was one of the top hits for incidental DLB in a recent network analysis study (Santpere et al., 2018). Although we cannot directly link this CNV to the development of DLB in these cases, it is interesting that a lysosomal enzyme is the top hit in our association analysis, given the prominent role of the lysosome in Lewy body diseases. In fact, a strong GWAS hit for DLB is GBA, a gene involved in lysosomal lipid storage disorders.

Also associated with DLB and absent from publicly available databases was a deletion overlapping the NME1 locus. NME1 is involved in purine metabolism, which has been reported to be disrupted in AD, PD, and Creutzfeldt–Jakob disease. NME1 mRNA was also found to be reduced in these diseases (Ansoleaga et al., 2015, 2016; Garcia-Esparcia et al., 2015). Here, we identified a deletion at the 3’-end of NME1, which could be consistent with a reduced expression of the gene in DLB, although this was not tested in the present study.

Using a candidate gene approach where we analyzed genes known to have a role in DLB and DLB-related diseases, we identified several CNVs of potential interest. The hallmark of DLB at autopsy is the accumulation of alpha-synuclein protein within neurons and their processes, termed Lewy bodies and Lewy neurites (Spillantini et al., 1997). Variants in the SNCA gene, which encodes alpha-synuclein, have been previously associated with the risk of developing DLB (Bras et al., 2014; Guerreiro et al., 2018). In addition to point mutations, CNVs including SNCA are known to cause PD, and over the past years, evidence has suggested that this gene may also be duplicated in DLB. Nishio et al. identified a PD family with a duplication spanning all of SNCA and MMRN1 where the proband was later neuropathologically diagnosed as DLB (Nishio et al., 2006; Obi et al., 2008). Four neuropathologically diagnosed DLB cases presented a large duplication from DSP to PDJM5 including SNCA, 3 of these were heterozygous and 1 was homozygous (Ikeuchi et al., 2008). A duplication in SNCA was also described in a probable DLB patient in a study with 99 cases (Meeus et al., 2012). Here, we add to this body of evidence, by identifying another patient neuropathologically diagnosed with DLB carrying a SNCA duplication. In our DLB cohort, this duplication shows a similar frequency to that provided by DGV. However, the frequency reported in DGV results from 2 entries in that database. When looking in more detail at these 2 entries, they are actually duplicated entries from the same Human Genome Diversity Project (HGDP) sample from Cambodia (HGDP00721). Given that information for each HGDP samples is limited to sex of the individual, population, and geographic origin, it is possible this sample originated from a PD or DLB patient or from an asymptomatic carrier, as these have previously been reported, with SNCA multiplications having particularly low penetrance levels in Asian populations (Ahn et al., 2008; Nishio et al., 2006). It is also possible the duplication reported is an artifact caused by the creation or passage of the lymphoblast cell lines used to extract DNA (Simon-Sanchez et al., 2007).

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**Fig. 1.** Schematic representation of statistically significant CNVs resulting from the case-control analysis that were not found in controls or publicly available databases. CNVs overlapping LAPTMS in A; and SPAG9/NME1 in B. Known transcripts are represented at the bottom of each panel. Passing QC SNPs used for the CNV calling are depicted by color according to the genotyping array (red: OmniExpress, blue: Omni2.5 M, green: Omni2.5 M for controls). CNVs (duplications) are depicted as bars above the genes. The gray shadow area represents the associated region that is genome-wide significant. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
We also identified one heterozygous duplication encompassing \textit{CHCHD10}, a gene previously shown to cause FTD and/or amyotrophic lateral sclerosis (Zhang et al., 2015). However, given that disease-associated \textit{CHCHD10} mutations are loss-of-function (Perrone et al., 2017), it is not clear whether a duplication of the gene would be pathogenic.

\textit{GABRB3} is a gamma-aminobutyric acid (GABA) receptor that was reported to be associated with DLB in a recent GWAS but did not survive independent replication (Guerreiro et al., 2018). However, loss of GABA receptors could underlie the typical visual hallucinations in DLB (Khundakar et al., 2016), and because of this, we specifically looked at CNVs in \textit{GABRB3} and identified a duplication in one case. Given the GWAS results previously mentioned, the CNV detected here and the fact that GABA receptor neurotransmission is altered in DLB (Santpere et al., 2018), it is tempting to speculate that genetic variability in GABA receptors may, in fact, modulate risk for altered in DLB (Santpere et al., 2018), it is tempting to speculate that genetic variability in GABA receptors may, in fact, modulate risk for DLB.

We identified 2 clinically diagnosed DLB samples with \textit{MAPT} duplications (Fig. 3). \textit{MAPT} was not found to be significantly associated with DLB in recent GWAS but did not survive independent replication (Bras et al., 2014; Guerreiro et al., 2018), but the \textit{MAPT} H1 haplotype was previously described as a possible risk factor for DLB (Cervera-Carles et al., 2016; Labbé et al., 2016) and is a well-known genetic risk factor for PD. Previous studies of small cohorts of FTD patients have not revealed causative \textit{MAPT} duplications (Lladó et al., 2007; Skoglund et al., 2009) but the screening of French FTD patients including multiplex families led to the identification of a heterozygous partial deletion of \textit{MAPT} (Rovelet-Lecrux et al., 2009) and a 17q21.31 microduplication in an atypical FTD case (Rovelet-Lecrux et al., 2010). More recently, \textit{MAPT} duplications were shown to increase expression of \textit{MAPT} mRNA and were found to cause tangle pathology without Aβ deposition in probable AD patients (Le Guennec et al., 2017).

\textit{PARK2} homozygous CNVs are the most common copy number cause of PD, accounting for more than 50% of all pathogenic mutations in the gene and more frequently affecting the region between exons 2 and 7 (Hedrich et al., 2004; Kim et al., 2012). Our results showed no significant differences in the frequency of heterozygous CNVs overlapping \textit{PARK2} between DLB cases and controls, similar to the findings by Kay et al. in PD (Kay et al., 2010). In addition, we did not find any homozygous \textit{PARK2} CNVs suggesting that CNVs in this gene do not play a causative role in DLB.

\textit{APP} duplications are known to cause AD (Delabar et al., 1987; Ghani et al., 2012; Swaminathan et al., 2011, 2012; Zheng et al., 2014, 2015). The sample carrying an \textit{APP} duplication in our cohort has a clinical diagnosis of DLB without neuropathological confirmation; it is therefore possible that this is an AD case misdiagnosed as DLB. However, there have been reports in the literature of DLB cases associated with \textit{APP} duplications. For example, in a French family presenting with a diverse phenotype, \textit{APP} duplication was associated with DLB confirmed by neuropathological findings (Guyant-Marechal et al., 2008). Similarly, one case with Lewy body–variant AD was reported in a multigenerational dementia family from the Netherlands (Sleegers et al., 2006). DLB cases frequently present Aβ pathology at autopsy (Hepp et al., 2016), and it has been suggested that Aβ accumulation can trigger Lewy body disease (Masliah et al., 2001).

There are 2 main limitations in this study: first, this is a relatively small-sized cohort, which means we cannot confidently assess associations of CNVs with low effect sizes on disease; second, we did not perform independent replication of these findings, which precludes us from establishing definite associations or causes of disease. Despite these limitations, we report on the first systematic analysis of CNVs in a large cohort of DLB patients, using well-established analytical practices. We identified potential disease causing CNVs as well as potential novel candidate genes for DLB. Despite this, our results suggest that it is unlikely that CNVs play a significant role in the pathogenesis of DLB.

5. Disclosure

The authors have no actual or potential conflicts of interest.

Acknowledgements

RG and JB work is funded by fellowships from the Alzheimer’s Society. TO work is funded by a scholarship from The Lewy Body Society. For the neuropathologically confirmed samples from Australia, tissues were received from the Sydney Brain Bank, which is supported by Neuroscience Research Australia and the University of New South Wales, and GMH is funded by an NHMRC senior principal research fellowship. The authors thank the South West Dementia Brain Bank (SWDBB) for providing brain tissue for this study. The SWDBB is supported by BRACE (Bristol Research into Alzheimer’s and Care of the Elderly), Brains for Dementia Research, and the Medical Research Council. The authors acknowledge the Oxford Brain Bank, supported by the Medical Research Council (MRC), Brains for Dementia Research (BDR) (Alzheimer Society and Alzheimer Research UK), Autistica UK, and the NIHR Oxford Biomedical Research Center. The brain samples...
Fig. 3. Duplications identified at the MAPT locus. Log R ratio and B allele frequency plots of the CNVs identified at the MAPT locus in 2 clinically diagnosed DLB cases. Each point represents an SNP according to location in chromosome 17 (position on X axis). The genomic duplication is indicated by an increase in log R ratio and B allele frequency clusters outside the expected values of 1 (B/B), 0.5 (A/B), and 0 (A/A). Genes are represented at the bottom as black bars. SNPs inside the CNV regions are represented in red, SNPs outside the CNV regions are represented in blue, and SNPs in MAPT are represented in green. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
variations reveals PARK2 as a candidate gene for attention-deficit/hyperactivity disorder. Mol Psychiatry 19, 115–121.


