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Genetic association and functional characterization of *MCPH1* gene variation in bipolar disorder and schizophrenia

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Abstract

A rare microcephalin 1 gene (*MCPH1*) variant rs61749465A>G (p.Asp61Gly) with prior evidence for association with schizophrenia ($p=3.78x10^{-7}$; Leonenko et al., 2017) was tested for association in 2,300 bipolar disorder (BPD) participants, 1,930 SCZ participants and 1,820 normal comparison subjects. We report evidence for association of rs61749465A>G with BPD (P=0.0009). rs61749465 is located in the N-terminal of the BRCT1 domain of MCPH1. Bioinformatic analysis predicted the Asp61Gly substitution to be damaging to MCPH1 function. A second *MCPH1* BRCT1 domain variant (rs199422124C>G; p.Thr27Arg), reported to cause autosomal recessive microcephaly, was not detected in the participants tested here. We sought to characterize the functional effects of these variants on *MCPH1* function. Cell count assays indicated that rs199422124 allele G had a greater impact on cell survival compared to the G allele of rs61749465. Gene expression analysis combined with gene network and pathway analysis indicated that rs61749465 allele G may impact protein translation and cell cycle control.

The evidence for association between rs61749465A>G and psychosis in both BPD and SCZ warrants further replication. Likewise, the data from the functional analyses point to molecular mechanisms that may underlie the proposed *MCPH1* mediated risk of psychosis and pathogenesis in autosomal recessive microcephaly require additional experimental validation.

Keywords

Allelic association; RNA sequencing; MCPH1; bipolar disorder; schizophrenia

1. Introduction

There is overlap in the symptoms of schizophrenia and bipolar disorder (BPD) and the clear distinction between SCZ and BPD proposed by Kraepelin is a subject of ongoing debate [Craddock and Owen 2010]. The association of the same genetic markers and genes with

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both BPD and SCZ has provided further evidence for the concept of a spectrum of psychotic disorders [Ferreira and others 2008; Green and others 2005; Nyegaard and others 2010; O'Brien and others 2014].

We have previously performed weighted burden analyses on SCZ exome sequencing data from the UK10K project and a Swedish case control sample to identify low frequency and rare variants associated with the disorder [Al Eissa and others 2017; Curtis and UK10K Consortium 2016; Purcell and others 2014; UK10K Consortium and others 2015]. Using this approach, we identified the *MCPH1* (microcephalin 1) gene as having a signed log P-value (SLP) of 3. The SLP is the base 10 logarithm of the P-value given a positive sign if the excess of variants is in cases. Four *MCPH1* variants that were observed to be twice as common in cases as controls including the low frequency variant rs61749465. We have also recently reported a nominal association between this variant in 10,011 SCZ cases and 13,791 controls [Leonenko and others 2017]. This study included data from our own University College London (UCL) SCZ case control sample.

Hemizygous *MCPH1* truncating variants and homozygosity of the rare *MCPH1* variant rs199422124 have been shown to be a cause of primary microcephaly with intellectual disability [Morris-Rosendahl and Kaindl 2015]. However, we could find no reports in the literature of individuals with MCPH1 derived microcephaly and psychosis.

The *MCPH1* gene is composed of 14 exons, which encode three BRCA1 (breast cancer type 1 susceptibility protein), C Terminus (BRCT) domains. The first domain, BRCT1, extends from amino acids 7 to 83, the second BRCT2 domain is from amino acid 642 to 720, and the third BRCT3 domain is from amino acid 753 to 823 [Jackson and others 2002]. *MCPH1* is also known as *BRIT1* (BRCT-repeat inhibitor of the transcriptional repressor of human telomere reverse transcriptase, hTERT) [Jackson and others 2002]. *MCPH1* has multiple roles, which include regulating DNA damage/repair pathways [Rai and others 2006]; centrosomal localization [Jeffers and others 2008]; E2F transcription factor 1-mediated apoptosis [Shi and Su 2012; Yang and others 2008]; transcriptional activation of cell cycle checkpoint genes and DNA stability [Passemard and others 2011]; and telomere structure, by binding to the proximal region of the hTERT promoter [Shi and Su 2012]. The rs199422124 variant leads to a change in amino acid charge and polarity. It is present in a highly conserved region of the gene and is reported to impact the regulation of the cell cycle [Trimborn and others 2006]. One of the mechanisms by which the rare variant may lead to microcephaly is through premature chromosome condensation (PCC), whereby chromatin condenses prematurely in the early G2-phase of the cell cycle [Trimborn and others 2006].

Here we report an investigation of the frequency of the G allele of rs61749465 in the UCL BPD sample and the frequency of the G allele of rs199422124 in both the UCL BPD and SCZ samples. We also went on to functionally characterize the *in vitro* effect of these variants and in particular we assessed the effect of these variant on gene expression because of *MCPH1*'s reported role in this process.

2. Methods

2.1. UCL Participant samples

All cases in our study had received a clinical diagnosis of SCZ or BPD according to the International Classification of Disease version 10 (ICD-10) and were interviewed by a psychiatrist or trained researcher using the lifetime version of the Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L) schedule to confirm the diagnosis [Spitzer 1977]. Case participants were also rated with the 90-item Operational Criteria Checklist (OPCRIT) [McGuffin and others 1991]. The UCL BPD and SCZ samples comprised 2300 and 1930 participants respectively. The BPD subjects were collected in several phases. The first phase (UCL1) comprises 506 subjects diagnosed with BPD-I [Sklar and others 2008]. For UCL1 all DNA was obtained from blood samples. Both the second and third phases (UCL2 and UCL3) included participants with BPD-II that were extracted either saliva or blood [Dedman and others 2012; Fiorentino and others 2015]

The UCL control sample consisted of 1820 volunteers, 1340 of whom were interviewed with the initial clinical screening questions of the SADS-L and selected on the basis of not having a past or present personal history of any Research Diagnostic Criteria (RDC) defined mental disorder. Heavy drinking and a family history of SCZ, alcohol dependence or BPD, were also used as exclusion criteria for controls. The remaining 480 DNA samples were unscreened healthy British volunteers collected by the European Collection of Animal Cell Culture (ECACC).

All cases and controls were of UK or Irish ancestry [Datta and others 2007]. UK National Health Service multi-centre and local research ethics approvals were obtained and all subjects gave signed informed consent.

2.2. Cell culture

Human embryonic kidney, HEK293 were used in this study, which despite their name, exhibit properties of neuronal lineage cells [Shaw and others 2002], cells were grown in Dulbecco's Modified Eagle's Medium (DMEM; Gibco) containing 10% FBS. Media was supplemented with 1% penicillin/streptomycin (Sigma), at 37°C in a humidified incubator with 5% CO_2 in air. Adherent cells were passaged at 70-80% confluence.

2.3. DNA extraction and quantification

Genomic DNA was obtained from frozen whole blood or saliva samples. DNA was extracted from blood samples using phenol-chloroform [Pereira and others 2011] and BACC-DNA Extraction kits (Illustra Nucleon Genomic, GE Healthcare, UK). Saliva samples were collected using Oragene kits (DNA Genotek, Ottowa, Canada) and DNA was extracted according to the manufacturer's instructions. All DNA samples were quantified by PicoGreen fluorimetry (Invitrogen, Paisley, UK).

2.4. Genotyping, sequencing and genetic analysis

Fluorescent allele-specific PCR genotyping assays were designed for the *MCPH1* variants (KASPar; LGC Genomics, Hoddesdon, UK). Genotyping was performed on a LightCycler

480 Real-Time PCR System (Roche Diagnostics, Burgess Hill, UK) in BPD, SCZ, and control samples. Validation of the genotypes in heterozygote individuals was performed with DNA sequencing (3730xl DNA Analyzer, Applied Biosystems, UK). Genotyping assays were validated by including DNA samples that carried the alternate allele for each variant tested on all of the genotyping plates. The assay for rs61749465 was validated by the inclusion of DNA from a BPD subject known to be heterozygous for the variant from whole genome sequence data generated in a subset of the UCL BPD cohort [Fiorentino and others 2015] and the assay for rs199422124 was validated using DNA from a cDNA clone carrying the variant allele (see below). Data were analyzed to confirm Hardy-Weinberg equilibrium and tests of allelic association were performed using a Fisher's exact test. A cut-off significance value of $P < 0.05$ was used.

2.5. Bioinformatic analysis

The potential functional consequences of *MCPH1* variants on the mRNA secondary structure and the MCPH1 protein was predicted using the UCSC genome browser (http://genome.ucsc.edu/), RNAfold (http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi [Gruber and others 2008], Mfold (http://mfold.rna.albany.edu/?q=mfold) [Zuker 2003], SIFT [Sim and others 2012], and PolyPhen2 [Adzhubei and others 2013].

2.6. DNA plasmid construction

To test the functional effects of the two *MCPH1* variants, the variant alleles for rs61749465 (G) and rs199422124 (G) were introduced in an expression vector containing human *MCPH1* (IOHx61411-pDEST26, Source BioScience, UK) using the QuickChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies, Stockport, UK). Oligonucletides were designed using the QuikChange Primer Design tool (www.genomics.agilent.com/primerDesignProgram.jsp). The G allele of rs61749465 was introduced using the following primers: forward (5'-CTCTCTTCTGAGCTTTGCCCCAAGTGCTCTGGTAG-3') and reverse (5'-CTACCAGAGCACTTGGGGCAAAGCTCAGAAGAGAG-3'); and the G allele of rs199422124 was introduced using the following primers: forward (5'-ATCCACAAGCTGTGTTGTAAATCTCTTTGAATAATTTTCTGTTCCA-3') and reverse (5'-TGGAACAGAAAATTATTCAAAGAGATTTACAACACAGCTTGTGGAT-3'). The sequences of all the experimental constructs were verified by DNA sequencing.

2.7. Transfection

HEK293 cells were seeded in 6-well plates prior to transient transfection with *MCPH1* reference sequence and with the variant alleles of rs61749465, rs199422124 using Lipofectamine® 2000 (Invitrogen, UK). Two negative control wells (untransfected cells) were added to the plate. Cells were cultured at 37°C for 48 hours at 5% CO_2 to reach 90% confluence. Stable cell line generation was performed by positive selection with the Geneticin (G418) (Sigma, UK) resistance marker. A dose-response kill curve was performed to choose the optimal concentration of the antibiotic that inhibited transiently-transfected HEK293 cell growth. G418 (0.4 mg/ml) was added to each well in culture media (see above) and the media was replaced every three days over two to four weeks in order to obtain a stably-transfected cell line.

2.8. Cell metabolic activity assays

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was performed to test the metabolic activity of cells expressing clones containing the variant alleles of rs61749465 and rs199422124 compared with the reference sequence *MCPH1* clone described above. Data shown represent triplicate wells from three independent experiments [Mosmann 1983].

2.9. Comet DNA damage assays

The Comet assay was used to measure the effect of *MCPH1* variants on DNA damage repair. The assay was performed on HEK293 cells stably-transfected with the reference *MCPH1* sequence clone, or clones containing the variant alleles for either rs61749465 or rs199422124. The cells were cultured for two weeks, before cells were embedded in 1% agarose on a pre-coated microscope slide. Comet assay was performed as described before [Hartley and others 2011]. Cellular DNA was stained with propidium iodide and DNA damage was quantified using Komet Analysis software 4.02 (Andor Technology, UK). Assays were performed on 50 cells for each condition in triplicate with the cells transfected with each variant allele, the reference clone and untransfected control cells and placed on each slide.

2.10. mRNA stability assays

Actinomycin D, an inhibitor of transcription, was used to determine the effect of *MCPH1* variants on mRNA stability. HEK293 cells stably transfected with the two *MCPH1* variant clones (rs61749465 and rs199422124) and the reference clone were plated in triplicate in six well-plates along with untransfected cells. With the exception of the 0 hour time point, cells were treated with 6.5μg/ml ActD (Sigma-Aldrich, UK), and incubated for time points of up to 24 hours, before RNA was extracted and reverse transcribed. Quantitative-PCR was used to examine the level of *MCPH1* mRNA at each time point (described below).

2.11. RNA extraction and reverse transcription

RNA was isolated using the RNeasy Mini Kit (Qiagen) and a DNase-I digestion step was included to eliminate residual DNA. The quantity of the RNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, UK). A complementary cDNA library was prepared from 5μg of total RNA using SuperScript III reverse transcriptase (RT) (Life technology, UK). RNA was incubated with 10 mM dNTP mix, 50 μM oligo(dT)15, in a final volume of 13 μl, heated to 65°C for 5 mins and then incubated on ice for 1 min. cDNA synthesis was prepared adding 1 μg of RNA preparation to 0.1 M DTT (dithiothritol), RNasin ribonuclease inhibitor (recombinant RNasin, Promega, UK) and 5 μl SuperScript III RT (200 U/μL) and 5X First-Strand Buffer. Samples were then incubated for 10 min at 25°C, followed by 50 min at 50°C. Reactions were terminated by incubation at 85°C for 5 min before being chilled on ice.

2.12. Quantitative PCR

SYBR Green Master Mix (Lightcycler 480 SYBR Green 1 Master, Roche, Germany), comprises 50% of the final 10 μl volume containing 10ng DNA template and 10 pmoles/ul

of each *MCPH1* forward and reverse primers (5'-AAGCTCGTTTCGGTGCTCT-3' and 5'-TTAGCTGCAGGGAACAATGA-3') (KiCqStart SYBR Green primers, Sigma, UK). Cycling conditions were 95°C for 10 min; 45 cycles of 95°C for 15 seconds, 60°C for 1 minute and 95°C for 15 sec. Expression of several housekeeping genes were measured (data not shown) but only that of *GAPDH* was unaffected by overexpression of *MCPH1* (primers sequences are in Supplementary Table 1). *MCPH1* gene expression relative to *GAPDH* was quantified using $2^{-\Delta\Delta D}$ equation [Livak and Schmittgen 2001].

2.13. RNA Sequencing

Total RNA was extracted as described above from HEK293 cells stably transfected with the reference and variant clones of *MCPH1* and from untransfected HEK 293 control cells that had been cultured in six-well plates. PolyA-selected mRNA libraries were prepared from the total RNA using the using the mRNA-Seq Sample Preparation Kit (Illumina, at Eurofins, Germany). 125 nucleotide single read RNA sequencing (RNAseq) was performed on an Illumina HiSeq v4 sequencer (Eurofins, Germany). Quality control (QC) analysis of the RNAseq data was performed using FASTQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and the NGS QC Toolkit [Patel and Jain 2012]. Adapter sequences were removed using Trimmomatic [Bolger and others 2014]. The RNAseq Analysis Pipeline (RAP) [D'Antonio and others 2015] was used to align the sequence reads to the human genome (build GRCh37), and to normalize and quantify read counts. Within RAP, Bowtie [Langmead 2010] was used to remove un-spliced reads and to map reads against the reference genome. Cufflinks version 2.2.1 [Trapnell and others 2010] was then used for transcript quantification and reconstruction; HTSeq [Anders and others 2014] STAR version 2 [Dobin and others 2013] was used for alternative mapping; and DESeq [Anders and Huber 2010] was used for differential expression analysis.

In order to identify genes whose expression levels were significantly altered by the presence of the *MCPH1* variant alleles compared with *MCPH1* reference clone, genes whose expression was significantly altered by over expression of either reference or variant forms of *MCPH1* were first removed. This was achieved by filtering genes whose expression was altered (unadjusted $P < 10^{-4}$) in the RNAseq data from either the *MCPH1* reference clone and/or the *MCPH1* variant clones compared with the untransfected cells. An FDR adjusted Q value of <0.05 was used to define genes whose expression was altered by the *MCPH1* variant clones (rs61749465 or rs199422124) relative to reference *MCPH1* clones and these genes lists were used for subsequent analyses.

Signaling Pathway Impact Analysis (SPIA) [Tarca and others 2009] implemented in Graphite Web http://graphiteweb.bio.unipd.it/ [Sales and others 2013] was used to perform pathways analysis on genes whose expression was altered (FDR < 0.05) in the presence of the *MCPH1* variant alleles compared to the reference clone. The analysis was performed using KEGG pathways and on the Reactome database. Gene networking analyses was performed using GENEMANIA (http://genemania.org/).

3. Results

3.1. Genotyping

The missense variant rs61749465 was genotyped in 4,205 UCL cases and 1809 control samples. The G allele variant was found to be associated with BPD (P = 0.0009) and SCZ (P = 0.0367) and with both diseases combined (P = 0.0024) (Table 1). The genetic association findings with SCZ have already been reported in Leonenko et al [2017]. The microcephaly associated-autosomal recessive variant allele (G) of rs199422124, was not detected in any of the cases or control subjects tested.

3.2. Bioinformatic analysis

MCPH1 rs61749465 is located on chromosome 8 at position 6272353 (hg19; NM_024596.4 c.182A>G (p.Asp61Gly) and rs199422124 is located on chromosome 8 at position 6409336 (hg19; NM_001322043.1 c.74C>G) (p.Thr27Arg). RNAfold and Mfold analysis indicated that neither the rs61749465 G allele nor the rs199422124 G allele likely to affect the mRNA structure compared to the reference sequence (supplementary figure 1). Both variants were described as deleterious on SIFT and possibly damaging according to PolyPhen2. The rs61749465 A>G substitution leads to an amino acid change from aspartate, which is an acidic polar charged residue, to glycine, a nonpolar and neutral amino acid at position 61 of the peptide in the BRCT1 domain of MCPH1. rs199422124 leads to an amino acid substitution at position 27 from threonine to arginine. This non-conservative amino acid change results in the replacement of a neutrally charged residue with a basic one.

3.3. Cellular analysis

We did not observe morphological changes in cells stably-transfected with either the *MCPH1* variant that was associated with BPD and SCZ (rs61749465) or the microcephaly-associated variant (rs199422124) compared to cells either transfected with the *MCPH1* reference clone or with untransfected cells. Mean differences in the data between groups was compared using analysis of variance to determine differences between groups. Overexpression of reference *MCPH1* led to a significant reduction in the cell counts compared to untransfected cells across all four time points (P=0.0015, P=0.0037, P=0.0063, and P=0.00038 for the 24, 48, 72, and 96 hour time points respectively). For the *MCPH1* rs61749465 variant allele (G) there was a significant increase in the number of cells relative to wild type only at the 96 hour time point (P=0.013; Supplementary Table 2). In contrast however for the rs199422124 *MCPH1* variant allele (G) cell counts were lower than those for the reference *MCPH1* transfected cells at the 24, 48 and 72 hour time points (P=0.046, P=0.00012, and P=0.013 respectively; Supplementary Table 2).

The *MCPH1* rs61749465 variant allele G led to a significant decrease in cell metabolic activity between 24 and 72 hours relative to wild type (24 hours P=0.018; 72 hours P=0.027; Supplementary Table 2). For rs199422124 variant allele G there was evidence for increased cell metabolic activity at 48 hours and at subsequent time points (48 hours P=0.0095; 72 hours P=0.0015; 96 hours P=0.015; Supplementary Table 2). The only evidence for an impact on cell metabolic activity from over expression of the reference *MCPH1* clone relative to the untransfected cells was at 72 hours (P=0.028; Supplementary Table 2).

3.4. DNA damage

Neither the *MCPH1* wild type, rs61749465 or rs199422124 variant alleles significantly affected DNA damage repair compared to untransfected HEK293 cells, as measured by the electrophoresis-based Comet assay (one-way ANOVA, Head DNA: F = 0.495, df =3, P = 0.696, n = 12; Tail DNA: F = 0.045, df =3, P = 0.696, n = 12; Kruskal-Wallis, Olive: χ^2 = 2.012, df = 3, P = 0.570, n = 12) (Supplementary Table 3).

3.5. mRNA stability

The analysis performed using Levene's test for homogeneity of variance was significant ($P \leq 0.05$), non-parametric Kruskal-Wallis analyses were performed (SPSS 24, IBM). Neither the presence of the rs61749465 or rs199422124 variant alleles significantly impacted the stability of *MCPH1* mRNA following actinomycin D treatment over the 24 hour time period compared with untreated cells at 0 hours (Kruskal-Wallis, rs61749465: χ^2 = 10.996, df = 6, P = 0.089, n = 21; rs199422124: χ^2 = 10.877, df = 6, P = 0.092, n = 21) (Data not shown).

3.6. RNA Sequencing

RNAseq analysis successfully mapped ~30 million single end reads (99.11-99.30%) to the human genome for each sample. The expression of 384 genes was significantly altered by over expression of the reference or variant clones of *MCPH1* compared to the untransfected cells and these genes were not considered further in the analyses. The analysis of mRNA from HEK293 cells stably expressing *MCPH1* clone harboring the variant allele of rs61749465 identified 267 genes that were up (136) or down (131) regulated by the presence of the variant allele (FDR <0.05; Table 2 and Supplementary Table 4) in comparison to cells with the reference allele clone. Amongst the top ten genes whose expression was most significantly altered by the rs61749465 variant allele there were two genes that were down regulated and eight genes that were up regulated.

The analysis of mRNA from HEK293 cells stably expressing the *MCPH1* clone harboring the variant allele (G) of rs199422124 identified 193 genes that were up (96) or down (97) regulated by the presence of the variant allele (FDR <0.05; Table 3 and Supplementary Table 5) in comparison to cells with the reference allele clone. Amongst the top ten genes whose expression was most significantly altered by the rs199422124 variant allele were five genes that were downregulated and five genes that were upregulated. The expression of 47 genes was altered by both variants and this included 20 genes that were up regulated and 27 genes were down regulated (Supplementary tables 4 and 5). Supplementary table 8 contains a summary of the biological processes and diseases that have been found to be associated with the genes reported in tables 2 and 3.

SPIA pathways analysis using KEGG with the FDR < 0.05 corrected data for *MCPH1* rs61749465 and rs199422124 did not show evidence for the involvement any pathways. However the analysis of the data for rs61749465 provided evidence for the involvement of nine pathways including Eukaryotic Translation Elongation, Peptide chain elongation, GTP hydrolysis and joining of the 60S ribosomal subunit, Eukaryotic Translation Initiation, Cap-dependent Translation Initiation, Translation, Nonsense-Mediated Decay, Nonsense

Mediated Decay Enhanced by the Exon Junction Complex and Mitotic G1-G1/S phases (Supplementary Tables 6 and 7).

GeneMANIA analysis of genes whose expression was altered by rs61749465 demonstrated that 50.7% of these genes were co expressed with each other (Supplementary table 4); 37.7% had a physical interaction; 5.5% had a predicted physical functional relationship; 2.5% were co-localized in the same tissue or location; 1.8% were in genes exhibiting a genetic interaction; 1.0% were in the same reaction pathway; and 0.3% shared protein domains. Equivalent analysis with genes whose expression was altered by rs199422124 indicated that 59.3% were co-expression together (Supplementary table 5); 17.0% had a physical interaction; 12.7% had a predicted physical functional relationship; 7.7% were co-localized in the same tissue or location; 0.6% were in genes exhibiting a genetic interaction; and 0.2% shared protein domains. GeneMANIA analysis also identified additional genes whose expression was altered by rs61749465 that interact with *MCPH1*.

4. Discussion

We have demonstrated a novel association between rs61749465 and BPD. We have also shown support for our previous finding of association between this locus and SCZ, suggesting a broader role of the gene in the etiology of psychosis [Leonenko and others 2017]. We observed a higher frequency of the rs61749465 risk allele in BPD (0.0026) compared to that in SCZ (0.0013) in our own sample although none of these frequencies was greater than that observed in Leonenko et al [2017] (0.0046). The low allele frequency of this variant makes it difficult to draw conclusions as to the relative importance of this variant in risk of BPD or SCZ. There is no reported GWAS evidence for *MCPH1* association with BPD or SCZ [Group 2011; Hou and others 2016; Schizophrenia Working Group of the Psychiatric Genomics 2014], however, the association finding reported here warrants replication in a larger sample.

The *MCPH1* gene, also known as *BRIT1*, has been shown to be associated with autosomal recessive primary microcephaly [Jackson and others 2002]. Overexpression of the *MCPH1* gene has been reported to inhibit uncontrolled lung cancer cell growth by promoting cell apoptosis and arresting the cell cycle in S and G2/M phase [Zhou and others 2016]. Here, we have found that overexpression of a clone containing the reference *MCPH1* sequence led to a decrease in cell counts. We observed a further reduction in cell counts for the cells transfected with the *MCPH1* rs199422124 G allele clone. In contrast we observed a modest increase in the cell counts for the cells transfected with the *MCPH1* rs61749465 G allele clone compared to the reference clone at the final time point. We did not find evidence that either the *MCPH1* rs61749465 or rs199422124 variant alleles were predicted to alter mRNA folding. Likewise, neither variant significantly affected DNA damage or repair, or the stability of *MCPH1* mRNA. The data from the MTT cell metabolic activity assays did not yield clear patterns of differential effects between the different clones.

Pathways analysis from the RNAseq data suggests that the variant allele of rs61749465 leads to changes in protein translation and to cell cycle control. rs61749465 is located in the conserved N-terminal BRCT1 domain of the MCPH1 protein. This BRCT1 domain is

important for centrosomal localization throughout the cell cycle [Jeffers and others 2008]. The rs61749465 variant allele is predicted to encode a glycine residue. This residue is more hydrophobic than the reference aspartate residue and this substitution may lead to disruption of hydrogen bonds or protein folding within the MCPH1 protein [Venselaar and others 2010].

The results from the GeneMANIA analyses of genes whose expression was altered by rs61749465 identified nine genes that were co expressed with *MCPH1* (Supplementary table 4). These genes have been shown to be involved in: stimulating cell proliferation / migration (S*TIP1* and *EGFL7*) [Chao and others 2013] [Yang and others 2016]; cell proliferation and cancer development (*ZFR*) [Zhao and others 2016]; axon guidance and in the development of peripheral and central nervous system (*SEMA6B*) [Chao and others 2013] [Collet and others 2004]; cellular metabolism by regulating nucleic acid aggregation (*SFPQ*) [Lee and others 2015]; translation elongation and neuronal process extension and survival (*EIF5A*) [Huang and others 2007]; control of translation termination (*GSPT1*) [Chauvin and Jean-Jean 2008]; the regulation of development and maintenance of stem cells (*CTR9*) [Nagaike and others 2011]. GeneMANIA analysis for genes whose expression was altered by rs199422124 included two genes Desmocollin 3 (*DSC3*) and Cadherin 13 (*CDH13*) that were co expressed with *MCPH1*. *DSC3* is a calcium-dependent glycoprotein found primarily in epithelial cells and plays a role in desmosome cell-cell junction and cell adhesion [Nuber and others 1996]; and *CDH13* is a negative regulator of neural cell growth and copy number variants affecting the gene have been implicated in autism [Sanders and others 2011].

Data from the cell based, pathways and co expression analyses point to rs61749465 having an impact on cell cycle control and changes in cellular proliferation. Likewise, reduced expression of the schizophrenia risk gene *TCF4* in a cortical progenitor cell model influenced the cell cycle and cellular proliferation [Hill and others 2017]. These data suggest that a common disease risk mechanism may exist at the *TCF4* and *MCPH1* loci.

There are limitations to the approaches used to assess the impact of the *MCPH1* variants. The overexpression of cDNA from *MCPH1* with and without the variant alleles in a transformed cell line is unlikely to model the impact of these variants in a fully physiologically relevant manner. Second, analysis of the RNAseq data demonstrated that the expression of at least one mitochondrial membrane protein gene (*LETMD1*) was altered by the variant *MCPH1* alleles and in turn may have impacted the reliability of the MTT assay.

The results presented here provide evidence for association between the *MCPH1* rs61749465 G allele and psychosis in both BPD and SCZ. The genetic findings presented here warrant further replication in BPD and SCZ. Likewise, the data from the functional analyses reported here suggest that future *in vivo* or *in vitro* and experiments using iPSCs or primary rodent neuronal cultures potentially combined with the use CRISPR approaches are likely to be required to provide further insight into the molecular mechanisms that underlie the proposed *MCPH1* mediated risk of psychosis and pathogenesis in autosomal recessive microcephaly.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

Tests of association with rs61749465 in bipolar disorder and schizophrenia.

Sample	Genotype Counts		Total	MAF[†]	P value[‡]
	AA	AG			
Control	1809	0	1809	0	
Bipolar disorder	2284	12	2296	0.0026	**0.0009**
Schizophrenia[§]	1921	5	1926	0.0013	**0.0367**
BPD+SCZ	4205	17	4222	0.0020	**0.0024**
Total	6014	17	6031		

[†]MAF Minor Allele Frequency

[‡]Tests of allelic associations were calculated using the Fisher's exact test

[§]Data previously reported in Leonenko et al.

Table 2.

Selected genes with altered mRNA expression following overexpression of the *MCPH1* rs61749465 G allele relative to the reference allele in HEK293 cells.

Gene Symbol	Gene Name	Fold Change	P value	Q value
TXNIP	Thioredoxin interacting protein	0.74	$6.22x10^{-28}$	$1.86x10^{-24}$
CHORDC1	Cysteine And Histidine Rich Domain Containing 1	1.34	$3.02x10^{-19}$	$4.51x10^{-16}$
TRPM8†	Transient Receptor Potential Cation Channel Subfamily M Member 8	1.17	$1.81x10^{-12}$	$1.80x10^{-09}$
ANKRD12†	Ankyrin Repeat Domain 12	1.24	$1.10x10^{-10}$	$8.15x10^{-08}$
HSP90AA1	Heat shock protein 90kDa alpha (cytosolic), class A member 1	1.15	$1.47x10^{-10}$	$8.15x10^{-8}$
HSPA1B	Heat shock 70kDa protein 1B	1.19	$1.64x10^{-10}$	$8.15x10^{-8}$
HSPH1	Heat Shock Protein Family H (Hsp110) Member 1	1.21	$6.50x10^{-10}$	$2.77x10^{-7}$
STIP1	Stress-induced-phosphoprotein 1	1.13	$6.97x10^{-9}$	$2.60x10^{-6}$
RPL10†	Ribosomal protein L10	0.90	$1.44x10^{-8}$	$4.77x10^{-6}$
MRVI1†	Murine Retrovirus Integration Site 1 Homolog	1.13	$1.60x10^{-8}$	$4.77x10^{-6}$

RNA sequencing (RNAseq) was performed on mRNA from HEK293 cells stably transfected with plasmids containing the MCPH1 rs61749465 or reference sequence. The ten genes with the most significant P values are shown. Fold change is the mean of data from three replicate experiments comparing the variant to the reference allele was. The unadjusted significance values (P value) and the false discovery rate (FDR) adjusted and Q values are shown.

†Genes affected by over expression of MCPH1 carrying the variant alleles of rs199422124 and rs61749465.

Table 3:

Selected genes with altered mRNA expression following overexpression of the MCPH1 rs199422124 G allele relative to the reference allele in HEK293 cells.

Gene Symbol	Gene Name	Fold Change	P value	Q value
ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif, 1	1.29	$9.62x10^{-33}$	$2.51x10^{-29}$
ZNF532	Zinc finger protein 532	0.74	$1.11x10^{-13}$	$1.46x10^{-10}$
PCDH9	Protocadherin 9	0.80	$8.60x10^{-13}$	$7.49x10^{-10}$
TRPM8†	Transient receptor potential cation channel, subfamily M, member 8	1.17	$1.34x10^{-11}$	$8.72x10^{-9}$
HSPB8	Heat shock 22kDa protein 8	1.25	$5.78x10^{-11}$	$3.02x10^{-8}$
MLH3	mutL homolog 3	1.28	$6.48x10^{-10}$	$2.82x10^{-7}$
SNRPB	Small nuclear ribonucleoprotein polypeptides B and B1	0.88	$5.05x10^{-9}$	$1.88x10^{-6}$
PQLC1†	PQ loop repeat containing 1	0.80	$1.18x10^{-8}$	$3.85x10^{-6}$
TXNL4A	Thioredoxin-like 4A	0.84	$3.23x10^{-8}$	$9.34x10^{-6}$
MRVI1†	Murine retrovirus integration site 1 homolog	1.13	$3.58x10^{-8}$	$9.34x10^{-6}$

RNA sequencing (RNAseq) was performed on mRNA from HEK293 cells stably transfected with plasmids containing the MCPH1 rs199422124 or reference sequence. The ten genes with the most significant P values are shown. Fold change is the mean of data from three replicate experiments comparing the variant to the reference allele was. The unadjusted significance values (P value) and the false discovery rate (FDR) adjusted and Q values are shown.

†Genes affected by over expression of MCPH1 carrying the variant alleles of rs199422124 and rs61749465.