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DISSERTATION

Molecular genetic and cytogenetic analyses of autosomal recessive primary microcephaly (MCPH): Mouse model, new locus and novel mutations

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Table of Contents

Zusammenfassung

Bei der autosomal rezessiven primären Mikrozephalie (MCPH) handelt es sich um eine fetale Entwicklungsstörung des Gehirns, die sich bereits zum Zeitpunkt der Geburt als Mikrozephalie manifestiert. Die bisher bekannten Gene sind in die Kontrolle des Centrosoms involviert. Mutationen in dem *MCPH1* Gen des Menschen, das für das Protein Mikrozephalin kodiert, führen zu primärer Mikrozephalie in Verbindung mit einem singulären zellulären Phänotyp: der vorzeitigen Chromosomenkondensation (engl. premature chromosome condensation, PCC). Mikrozephalin weist zwei hoch konservierte BRCT Domänen auf und spielt eine wichtige Rolle in der Regulation des Zellzyklus, der Chromosomenkondensation und der DNA Reparatur. Im Rahmen dieser Promotion wurde das erste Mausmodell mit einem Defekt im *Mcph1* Gen generiert, das mit einer fehlerhaften Chromosomenkondensation einhergeht. Zudem wurde ein neuer *MCPH* Lokus auf Chromosom 10q11.23-21.3 kartiert sowie eine funktionell bemerkenswerte missense Mutation im *MCPH1* Gen, p.Trp75Arg, beschrieben, die die sich negativ auf die BRCT Domäne auswirkt. Ferner wurden drei Mutationen in dem *MCPH5* (*ASPM*) Gen identifiziert, p.Arg3096X, p.Arg1019X und die erste missense Variante p.Lys1862Gln. Insgesamt haben die Ergebnisse weitere Belege für die grundlegende Rolle der *MCPH* Gene für die Gehirnentwicklung geliefert.

Abstract

Autosomal recessive primary microcephaly (MCPH) is a neurodevelopment disorder, arising from a failure in embryonic neurogenesis, leading to microcephaly at birth. All known genes implicated in this disorder are involved in the monitoring of centrosome integrity. Mutations in the human gene *MCPH1,* encoding *microcephalin* protein, cause primary microcephaly associated with a unique cellular phenotype with premature chromosome condensation (PCC). *Microcephalin* contains highly conserved BRCT domains and has fundamental roles in cell cycle, chromosome condensation and DNA repair. The PhD project included generation of the first mouse model of impaired *microcephalin* function displaying the cellular phenotype of misregulated chromosome condensation, mapping a candidate MCPH locus on chromosome 10q11.23-21.3, reporting on a functionally notable *MCPH1* missense mutation, p.Trp75Arg, with destabilizing effect on BRCT domain and identification of three mutations in the *ASPM* gene for MCPH disorder type five: p.Arg3096X, p.Arg1019X and the first reported missense variant p.Lys1862Gln. These results together provide further biological evidence for the MCPH proteins essential roles in brain development.

Introduction

Autosomal recessive primary microcephaly (MCPH) is a rare and genetically heterogeneous disease due to impaired embryonic neurogenesis. Clinically, MCPH is defined by an OccipitoFrontal Circumference (OFC) < -3 SD at birth, a reduced brain volume, mental retardation and no further neurological findings except mild seizures [1, 2]. MCPH displays genetic heterogeneity with eight loci mapped to date [3-11]. The birth prevalence of the MCPH has been estimated to be 1.3 to 150 per 100,000 live-births, depending on the population and on the applied SD threshold to define the microcephaly. The eight respective loci and genes identified for this disorder include: MCPH1 (MIM ID #607117) encoding *microcephalin* [12], MCPH2 (MIM ID #604317) encoding *WDR62* [13], MCPH3 (MIM ID #604804) encoding *CDK5RAP2* [14], MCPH4 (MIM ID #604321) encoding *CEP152* [15], MCPH5 (MIM ID #605481) encoding *ASPM* [16], MCPH6 (MIM ID #609279) encoding *CENPJ* [14], MCPH7 (MIM ID #181590) encoding *STIL* [6] and the recently identified gene CEP63 encoding the *centrosomal protein 63* [11]. Mutations in the *ASPM* and *WDR62* have been suggested to be the most common causes of this disorder [17]. Further genetic heterogeneity likely exists as about 20–30% of MCPH families were shown to have no linkage to any of the known loci [18]. In fact, all eight known gene products are surprisingly localized to the centrosome suggesting that a common centrosomal mechanism is responsible for controlling the neuron number in the developing human brain [6, 14, 15, 19-21]. Human *MCPH1* gene is located on chromosome 8: 6,264,113-6,501,144 encoding *microcephalin* protein (UniProt ID Q8NEM0) that has three BRCT domains; one N-terminal BRCT domain at 1 – 93 AA and two C-terminal BRCT domains at 640 – 730 AA and at 751 – 833 AA. The hallmark of mutations in *microcephalin* is a unique cellular phenotype with a high proportion of cells with prophase-like chromosomes due to premature chromosome condensation in the early G2 phase of the cell cycle and delayed decondensation in the G1 phase. A model system with misregulated chromosome condensation will be useful to obtain further insights into the molecular mechanisms shaping the mitotic chromosomes.

Aims of the PhD thesis

1. Characterization of the cellular phenotype of the *microcephalin* deficiency in man and in mouse.
2. Identification of new locus for the MCPH disorder.
3. Identification of novel mutations within the known MCPH genes.

Hypotheses

1. Mouse *microcephalin*, particularly its BRCT domains, is required for unperturbed regulation of chromosome condensation.
2. In addition to the known genetic loci, further loci are responsible for the MCPH phenotype.
3. Missense mutation within the highly conserved N-terminal BRCT domain can disrupt the human *microcephalin* function.

Methods

Establishment of the Mcph1 mouse model

The model was generated using a mouse embryonic stem (ES) cell line from the BayGenomics (RR0608) containing a gene trap in intron 12 of the *Mcph1* gene (GeneID 244329) deleting the C-terminal BRCT-domain of the protein. Gene trap insertion site was identified with long range PCR (Roche) and gel electrophoresis. ES cells microinjections and mouse breeding were performed in the Max Planck Institute for Molecular Genetics, Berlin. Quantitative PCR reactions were carried out using the POWER SYBR GREEN PCR Master Mix (Applied Biosystems) and Applied Biosystems 7500 Real-Time PCR System according to the manufacturer's recommendations. Relative quantification was performed by the ΔΔCt method. DNA extraction from the mouse tail tips was done with chloroform or with PEQLAB lysis buffer. Mice genotypes were identified by PCR and gel electrophoresis. Forward and reverse primers flanking the trap integration site were applied for detection of the wild type allele. A reverse primer specific for the trap vector and a forward primer from the 5'-integration site were used to identify the trapped allele. Fibroblast cultures were established from the mouse tail tips or peritoneum using 0.5% Trypsin/EDTA and 10% collagenase/DMEM and maintained in DMEM supplemented with 15% fetal calf serum. Chromosome preparations and PLC assays were performed for the fibroblast cultures using the standard procedures. Cell cycle progression was studied with autoradiography by ^{3}[H]-Thymidine pulse labelling (1 μCi/ml). Chromosome decondensation was assessed with treatment of the fibroblast cultures with Cytochalasin B. Mice Brain MRI images were acquired using a 7 Tesla Bruker rodent scanner in the Charité Hospital Berlin. PCR, sequencing, immunofluorescence, western blot and X-gal staining experiments were performed following the standard protocols [22].

Genetic mapping for Autosomal Recessive Primary Microcephaly (MCPH)

Linkage to any of the known MCPH loci was first excluded using the STR markers. Touchdown PCRs were carried out to amplify the markers with 5'FAM labelled forward primers. Fragment analysis was performed with ROX 400 size standard marker on ABI 3100 DNA analyzer (Applied Biosystems) using the GeneScan/GeneMapper software. Genotyping was performed with the Human Mapping 10K Array (Affymetrix at RZPD, Berlin). For the homozygosity mapping, data was first converted into the appropriate format using Alohomora and Mega2 software. Parametric linkage analysis was performed with Merlin program for a rare recessive model. Haplotypes in the region of interest were estimated by Simwalk2 and visualized by HaploPainter. PCR sequencing was performed following the lab instructions [23].

Mutation screening of MCPH genes

For mutation detection, all 14 exons and exon–intron boundaries of the *MCPH1* gene (NM_024596) were PCR amplified. PCR products were sequenced and analyzed on ABI 3730 DNA analyzer using the Sequencing Analysis Software (Applied Biosystems). Sequence variant nomenclature was checked with the Mutalyzer program. Functional effects were predicted with MutationTaster, Polyphen and SIFT programs. Alignment of BRCT domains was assessed with ClustalW [24]. Lymphoblastoid cell lines (LCLs) were established from the mutation carrier cells as previously described (Neitzel 1986) [25]. Sequencing reactions were also performed for the entire coding regions of the *ASPM* gene (NM_018136/10434bp) using the Big Dye Terminator Ready Reaction Mix (V3.1 from Applied Biosystems). Thermocycler setting was 25 cycles of 96°C for 10sec, 50°C for 5sec and 60°C for 4min [26].

Chromosome condensation assay of MCPH1 patients' cells

Conventional cytogenetic analyses of cultured T-lymphocytes and lymphoblastoid cell lines (LCLs) were performed using standard techniques. 2000 nuclei were counted and the percentage of prophase/prophase-like and metaphase nuclei was calculated among total nuclei.

Results

Characterization of the Gene Trap in the ES Cell Line RR0608

The insertion of the vector into an intron of a gene results in a fusion transcript that joins the sequence of the exon, lying 5' to the insertion site, and the vector b-geo reporter, resulting in a truncated traceable fusion protein. In the ES cell line RR0608, the gene trap vector was inserted between exons 12 and 13 of the *Mcph1* gene, resulting in a truncated protein lacking 97 amino acids in the most C-terminal BRCT-domain. The fusion transcript was characterized by RT-PCR using gene and trap-specific primers and confirmed by sequencing. It was also crucial to identify the genomic insertion site, as the gene *Angpt2* resides in intron 12 of the *Mcph1* gene on the opposite strand. For this purpose we subdivided the intron (intron size is 99,179 bp) into sections of approximately 5,000 bp. We then performed long range PCR using sequence specific forward primers for each interval and a common trap-specific reverse primer. PCR using forward primer P19 resulted in the amplification of a 1,500 bp product. Subsequent sequencing of the PCR product determined the insertion site of the trap at position Chr8:18,778,752 of the mouse genomic sequence (version July 2007), therefore in a distance of approximately 37 kb of exon 1 of the *Angpt2* gene. Quantitative real time PCR confirmed that the expression of the *Mcph1* wild type transcript is markedly reduced in different tissues of the *Mcph1*$^{gt/gt}$ mice. Obviously, however, alternative splicing allows a strongly reduced but still detectable expression of the wild type allele. Residual wild type protein in the *Mcph1*$^{gt/gt}$ mice might be able to exert some function, however with significantly diminished activity due to its reduced abundance. In addition, even the trap-allele may adopt some *Mcph1*-functions because the gene trap truncates only the C terminus of the protein. Therefore, we intended to further characterize the expression from both the wild type- and trap allele. We were not able to detect the wild type *microcephalin* protein by western blotting neither in protein samples from the wild type nor from the *Mcph1*$^{gt/gt}$ animals. Also the b–geo reporter was not traceable in *Mcph1*$^{gt/gt}$ neither by X-gal staining nor by anti-b–galactosidase antibodies. This was explained later by very low expression level of the *Mcph1* [22].

Phenotypic Description of the Mice

Mcph1$^{gt/gt}$ animals did not differ significantly in body weight compared to wild type animals (P=0.193). Equally, the proportionate weight of the brains of *Mcph1*$^{gt/gt}$ animals was not reduced as compared to wild type (P =0.275). Measurement of brain volumes by MRI supported these findings. The average volume of *Mcph1*$^{gt/gt}$ brains (n =11) was 522.5 mm^3 ±22.8 compared to 514.4 mm^3 ±10.4 in wild type animals (n= 4). Moreover MRI analyses did not reveal any

obvious malformation of the brain. Most importantly however, the overall survival was reduced for the *Mcph1*$^{gt/gt}$ animals compared to wild type and heterozygous animals. Twenty-one *Mcph1*$^{gt/gt}$, seventeen heterozygous and fifteen wt animals were maintained under standard conditions to examine survival. The difference in survival developed after animals reached an age of >65 weeks and was statistically significant for the comparison of *Mcph1*$^{gt/gt}$ and wild type animals (log rank test χ^2= 4.1818, P= 0.0409) as well as the comparison of *Mcph1*$^{gt/gt}$ and heterozygous animals (log rank test χ^2= 10.1002, P= 0.0015). Unfortunately, we could so far not determine the precise causes of deaths for the *Mcph1*$^{gt/gt}$ animals [22].

Analyses of Chromosome Condensation

Primary fibroblast cultures from eleven *Mcph1*$^{gt/gt}$ mice and twelve wild type animals were established from the mouse tails and propagated under standard conditions. Cytological preparations were made to analyse chromosome morphology. Slides were coded and the fraction of prophase-like cells (PLCs) was determined by visual cell counts. 2000 nuclei were counted from each sample and the proportion of nuclei with prophase-like morphology and the metaphase indices were determined. *Mcph1*$^{gt/gt}$ cell lines showed a dramatic increase of nuclei displaying condensed prophase-like chromatin (7.7%±0.8) compared to wild type cell lines (1.0% ±0.5, χ^2 = 1306.49, p <0.001). In contrast, the metaphase indices were at the same level: 0.6% ±0.3 and 0.7% ±0.2 for *Mcph1*$^{gt/gt}$ and wild type cell lines, respectively. The similar metaphase indices confirm similar proliferative activities in gt/gt and wt/wt cell cultures. Quantitative analyses in 5 heterozygous cell lines revealed no increase in the proportion of prophase-like cells (1.22% ±0.5), an observation arguing against a potential dominant-negative effect of the gene trap allele.

We determined the timing of chromosome condensation following S phase by ^{3}[H]-thymidine pulse labeling. For this purpose, we compared one logarithmically growing diploid fibroblast cell line and one transformed cell line from *Mcph1*$^{gt/gt}$ mice to wild type cell lines in 1h intervals (1h–4h) by autoradiography after pulse-labeling with ^{3} [H]-thymidine for 10 min. N≥100 prophases or prophase-like cells were scored per time point and per cell line and the proportion of labeled cells was determined. We observed a significant difference between the gt/gt and wt/wt cell lines in the proportion of prophase/prophase-like cells that were labeled within this period. Already one hour after the pulse, 12% of the prophase-like cells of the diploid gt/gt mouse but none of the prophase cells in the control showed ^{3}H-thymidine labeling. After two hours, 42% of PLCs in the diploid *Mcph1*$^{gt/gt}$ cells and 12% of the prophases in wt cells were labelled. Similar results were obtained for the transformed cell lines. One hour after the pulse 35% of PLCs were labeled in the

Mcph1$^{gt/gt}$ cell line compared to only 12% in the wild type. Consequently, chromosome condensation in the *Mcph1*$^{gt/gt}$ cells starts in the early G2 phase of the cell cycle as soon as one hour after the end of the S-phase or even earlier. Thus, the observed premature chromosome condensation phenotype described in the cells of human patients with *MCPH1*-deficiency is also found in mouse cells homozygous for the trap-allele. To investigate the chromosome decondensation behavior of the *Mcph1*$^{gt/gt}$ cell lines in the early G1 phase of the cell cycle, we treated three logarithmically growing *Mcph1*$^{gt/gt}$ fibroblast cell lines and three wild type fibroblast cell lines with the inhibitor of cytokinesis, Cytochalasin B. Treatment with Cytochalasin B results in binucleated cells. Cytological preparations were performed following two hours of Cytochalasin treatment. Consequently, all binucleated cells must have exited mitosis within these two hours and, therefore, be in early G1 phase. 19.8% (± 2.7) of binucleated *Mcph1*$^{gt/gt}$ cells displayed condensed chromatin, while only 2.8% (± 1.2) of wild type binucleated cells showed signs of chromatin condensation (χ^2 =84.32, p<0.001). Thus, there is a delayed decondensation in *Mcph1*$^{gt/gt}$ cells. In summary, these results confirm a defective chromosome condensation in the *Mcph1*$^{gt/gt}$ cells similar to *MCPH1*-deficiency in human [22].

Also, in the chromosome preparations of the lymphoblastoid cells from the patient bearing the p.Trp75Arg mutation, we observed an extremely elevated fraction of PLCs, almost 8% of the cells, comparable to that of patients with truncating mutations. As already demonstrated for the patients with *microcephalin* truncating mutations, chromosome preparations of this patient revealed the characteristic poor metaphase resolution of not more than 300 bands per haploid genome in routine chromosome preparations [24].

New locus for Autosomal Recessive Primary Microcephaly (MCPH)

A consanguineous family of Turkish origin with four children, three of them affected with congenital microcephaly, was ascertained. The parents were first cousins. The microcephaly in the two older siblings remained continuously at about –3 SD since the first examination at birth while it increased with age in the youngest child from -4.3 SD at birth to –7 SD at the age of 4 years. The youngest patient also suffered from complex focal, partially generalized epileptic seizures at the age of four years. A MRI study showed bilateral parietooccipital small gyri, but no further abnormalities. Microcephaly of this patient was already noted during pregnancy in the 24th week of gestation by ultrasound examination. Cerebral MRI at four years displayed a thickened cortex and polymicrogyria. In all patients ultrasound examinations of heart and abdomen showed normal results as well as ophthalmologic and hearing evaluation. They did not

suffer from frequent infections. The personality of all patients was friendly with good social contacts [23].

Linkage to any of the known MCPH loci was excluded in the family with genotyping of the known loci microsatellite markers. This indicated that another, currently unknown, locus must be responsible for the clinical phenotype. Therefore, a genome wide SNP homozygosity mapping was performed using the Affymetrix Human Mapping 10K Array for all six members of the family. Parametric linkage analysis for a rare recessive model identified a common homozygous region of ~ 15.8 Mb in the chromosomal region 10q11.23-21.3 in the three affected patients. Linkage analyses showed the highest LOD score (2.83) for this region, defined by the flanking SNP markers rs1373791 and rs1880066. Haplotypes for 10 informative SNP markers on 10q11.23-21.3 were constructed using SIMWALK2, which confirmed the most likely candidate region between markers rs1373791 and rs1880066. This result was also confirmed by microsatellite genotyping of the family members with several markers from the candidate region. The homozygous region which should contain a novel gene for the primary microcephaly harbours a total number of 67 genes and 28 pseudogenes. The common feature of all MCPH genes identified so far (*MCPH1*, *WDR62*, *CEP152*, *CDK5RAP2*, *ASPM*, *CENPJ, STIL* and *CEP63*) is their involvement in centrosome and/or spindle organization and function. Therefore, genes encoding proteins that were reported to be involved in either mitosis, regulation of the cell cycle or in neuronal development and/or function were chosen as candidate genes and sequenced at the Institute of Human Genetics, Berlin. Although the underlying genetic defect was not identified, a possible molecular function in chromosome segregation was described in parallel by the characterization of mitosis in patients' cells [23].

Characterization of mutations in the MCPH genes

Microcephalin missense mutation p.Trp75Arg

An MCPH affected boy was referred for diagnostic evaluation at the age of 6 ¾ years because of severe microcephaly and developmental delay. He was the second child of consanguineous Turkish parents. He was born at 40 weeks of gestation with birth weight 2990g (-2.2 SD), length 52 cm (-0.7 SD) and head circumference 30.5 cm (-5.7 SD). The Apgar scores were 6/9/9. Early milestones were in the normal range. At age 6 ¾ he presented with a length of 117 cm (10th percentile) and head circumference 46 cm (-4.8 SD). Patient was remarkably pleasant with good social contacts.

Sequencing of genomic DNA revealed a homozygous missense mutation c.223T>C in exon 3 of the *MCPH1* gene. The resulting amino acid substitution tryptophan to arginine (p.Trp75Arg) changes highly lipophilic non-polar residue (Trp) to positively charged polar residue (Arg) in the critical α3-helix of the N-terminal BRCT domain. The nucleotide alteration was heterozygous in both parents. The c.223T>C alteration was absent from 180 alleles of ethnically matched controls indicating that this is not a common sequence polymorphism. Residue p.Trp75 is strictly conserved in *microcephalin* from Drosophila to Human. The program MutationTaster predicts the variant as disease causing (99.54% probability) and SIFT classifies that as a variant affecting the protein function. By PolyPhen the effect of p.Trp75Arg mutation was predicted as probably damaging with a PSIC score of 3.757 (score >1.7 is considered damaging), the highest among currently known MCPH1 missense mutations (p.T27R 1.912; p.H49Q 2.532; p.V50G 2.185; p.I51V 0.119; p.S72L 1.998). ClustalW alignment of BRCT domains for MCPH1 and BRCA1 shows that the highly conserved MCPH1 Trp75 residue is analogous to Trp1718 in the N-terminal BRCT domain and to Trp1837 in the C-terminal BRCT domain of BRCA1. Both, Trp1718 and Trp1837, are residues in the BRCA1 Trp-X-X-X-Cys/Ser motif while several missense alterations have been described for them in the Breast Cancer Information Core (BIC) Database [24]. *Microcephalin* residues p.H49 and p.I51, the previously reported mutated residues, are in ≤5 angstroms physical neighbourhood of the p.Trp75 in the N terminal BRCT domain. The arrangement of these residues might then maintain the packing of the *microcephalin* BRCT fold, which then can be disrupted by the missense mutations targeting them in the N-terminal BRCT domain in a way similar to BRCA1 missense mutations [24].

ASPM mutations p.Arg3096X, p.Arg1019X and p.Lys1862Gln

Sequencing of the *ASPM* gene revealed the underlying mutations in three MCPH families; p.Arg3096X (c.9286C>T), p.Arg1019X (c.3055C>T) and a missense mutation, p.Lys1862Gln (c.5584A>C). Sequencing of the *ASPM* gene was performed after previous localization of the MCPH5 locus with homozygosity mapping. The novel *ASPM* missense change (c.5584A>C; p.Lys1862Gln) in exon 18 of the *ASPM* gene is the first missense mutation described for the *ASPM* gene and converts the conserved basic amino acid lysine to neutral glutamine. Although predicted as benign variant by the Polyphen program (PSIC score 1.283), lack of this mutation in the healthy siblings and in the ethnically matched control subjects (studied at MPI for Molecular Genetics, Berlin) suggests that this missense change might disrupt the function of the protein [26].

Discussion

We established the first mouse model of impaired *Mcph1* based on an embryonic stem cell line from BayGenomics (RR0608), with a gene trap in intron 12 of the *Mcph1* gene, deleting the C-terminal BRCT-domain of the protein. Cell cultures generated from the mouse tissues bearing the homozygous gene trap mutation display the cellular phenotype of misregulated chromosome condensation, which is characteristic for the human disorder confirming defective *microcephalin* function. Furthermore, the overall survival rates of the *Mcph1*$^{gt/gt}$ animals were significantly reduced compared to wild type and heterozygous mice. *Mcph1*$^{gt/gt}$ animals showed no obvious physical abnormality and no reduced fertility [22]. This mouse model was also extensively studied with several assays in parallel, such as chromosome breakage analysis, immunoflouresence, flow cytometry, study of meiotic chromosomes, expression profiling and two-dimensional gel electrophoresis [22]. However, despite the phenotype of premature chromosome condensation (PCC) in the *Mcph1*$^{gt/gt}$ mice, no significant difference was observed between the cells/tissues of the wild type and *Mcph1*$^{gt/gt}$ animals for their response to DNA damage, foci formation and male meiosis as well as no specific patterns of differentially regulated genes was revealed in the *Mcph1*$^{gt/gt}$ animals. Compared to the brains of mouse models of Huntington's, Alzheimer's and Parkinson's disease, less numerous protein isoforms were altered in the proteomic analysis of the *Mcph1*$^{gt/gt}$ mice, which probably correlates with the very mild phenotype of *Mcph1*$^{gt/gt}$ mice [22].

We also described a candidate genetic locus in a consanguineous MCPH family. Microsatellite analyses revealed that the disorder did not map to any of the known MCPH loci. We performed genetic mapping using the SNP array genotyping data that revealed a homozygous region of 15.8 Mb with a LOD score of 2.83 for this disorder. The homozygous region was on chromosome 10q11.23-21.3, between markers rs1373791 and rs1880066 (chr10:49,783,988-65,594,216/hg19) and most likely harboring the underlying gene defect [23]. Although we were unable to identify the underlying genetic defect, analyses of chromosome nondisjunction in T-lymphocytes and fibroblasts performed in parallel [23] revealed a significantly elevated rate of nondisjunction in the patients' cells as compared with the controls. A remarkable alteration in the anaphase distribution of Aurora B and INCENP was also found confirming a chromosome segregation defect in patients. We did not observe, however, any other alteration regarding the cell cycle progression, chromosome structure or response to DNA damage [23].

Furthermore we reported on a novel *MCPH1* missense mutation, p.Trp75Arg, in the N-terminal BRCT domain of *microcephalin* associated with severe congenital microcephaly. Mutated residue belongs to an entirely conserved domain in the *microcephalin* orthologs of all vertebrate

species and Drosophila. Proliferating lymphocytes of the patient with this mutation showed the unique cellular phenotype of premature chromosome condensation indicating that this missense alteration disrupts the function of *microcephalin* N-terminal BRCT domain. *Microcephalin* residue p.Trp75 is tightly connected via hydrogen bonds with residues p.Ser72, p.Lys78 and p.Cys79. Interestingly, we observed in parallel that the residue p.S72 was mutated in another MCPH patient [24]. Both residues, p.Ser72 and p.Trp75, are strictly conserved in the BRCT domains of BRCA1. Missense alterations of the corresponding residues in BRCT domain of BRCA1 were previously described and predicted to be deleterious resulting in the destabilization of the BRCA1 protein. Our data provides further evidence for the functional significance of the highly conserved residues in BRCT domains [24].

Finally, sequencing of the *ASPM* gene in three MCPH families revealed two stop mutations and one missense mutation associated with the disorder confirming the key role of this protein in brain development [26].

These findings could help to better understand the genetics of the primary microcephaly and its underlying molecular mechanisms.

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PLoS One. 2010 Feb 16;5(2):e9242. doi: 10.1371/journal.pone.0009242.

Establishment of a mouse model with misregulated chromosome condensation due to defective Mcph1 function.

Trimborn M, Ghani M, Walther DJ, Dopatka M, Dutrannoy V, Busche A, Meyer F, Nowak S, Nowak J, Zabel C, Klose J, Esquitino V, Garshasbi M, Kuss AW, Ropers HH, Mueller S, Poehlmann C, Gavvovidis I, Schindler D, Sperling K, Neitzel H.

Institute for Medical Genetics, Charité-Universitätsmedizin Berlin, Berlin, Germany.

Abstract

Mutations in the human gene **MCPH1** cause primary microcephaly associated with a unique cellular phenotype with premature chromosome condensation (PCC) in early G2 phase and delayed decondensation post-mitosis (PCC syndrome). The gene encodes the BRCT-domain containing protein microcephalin/BRIT1. Apart from its role in the regulation of chromosome condensation, the protein is involved in the cellular response to DNA damage. We report here on the first **mouse** model of impaired **Mcph1**-function. The model was established based on an embryonic stem cell line from BayGenomics (RR0608) containing a gene trap in intron 12 of the **Mcph1** gene deleting the C-terminal BRCT-domain of the protein. Although residual wild type allele can be detected by quantitative real-time PCR cell cultures generated from **mouse** tissues bearing the homozygous gene trap mutation display the cellular phenotype of misregulated chromosome condensation that is characteristic for the human disorder, confirming defective **Mcph1** function due to the gene trap mutation. While surprisingly the DNA damage response (formation of repair foci, chromosomal breakage, and G2/M checkpoint function after irradiation) appears to be largely normal in cell cultures derived from **Mcph1**(gt/gt) **mice**, the overall survival rates of the **Mcph1**(gt/gt) animals are significantly reduced compared to wild type and heterozygous **mice**. However, we could not detect clear signs of premature malignant disease development due to the perturbed **Mcph1** function. Moreover, the animals show no obvious physical phenotype and no reduced fertility. Body and brain size are within the range of wild type controls. Gene expression on RNA and protein level did not reveal any specific pattern of differentially regulated genes. To the best of our knowledge this represents the first mammalian transgenic model displaying a defect in mitotic chromosome condensation and is also the first **mouse** model for impaired **Mcph1**-function.

PMID: 20169082 [PubMed - indexed for MEDLINE] PMCID: PMC2821930 **Free PMC Article**

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Publication Types, MeSH Terms, Substances

Cell Cycle. 2011 Sep 1;10(17):2967-77. Epub 2011 Sep 1.

Misregulation of mitotic chromosome segregation in a new type of autosomal recessive primary microcephaly.

Marchal JA, **Ghani M**, Schindler D, Gavvovidis I, Winkler T, Esquitino V, Sternberg N, Busche A, Krawitz P, Hecht J, Robinson P, Mundlos S, Graul-Neumann L, Sperling K, Trimborn M, Neitzel H.

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Abstract

Primary autosomal recessive microcephaly (**MCPH**) is a congenital disorder characterized by a pronounced reduction of brain size and mental retardation. We present here a consanguineous Turkish family clinically diagnosed with **MCPH** and without linkage to any of the known loci (MCPH1-MCPH7). Autozygosity mapping identified a homozygous region of 15.8 Mb on chromosome 10q11.23-21.3, most likely representing a new locus for **MCPH**. Although we were unable to identify the underlying genetic defect after extensive molecular screening, we could delineate a possible molecular function in chromosome segregation by the characterization of mitosis in the patients' cells. Analyses of chromosome nondisjunction in T-lymphocytes and fibroblasts revealed a significantly elevated rate of nondisjunction in the patients' cells as compared to controls. Mitotic progression was further explored by immunofluorescence analyses of several chromosome and spindle associated proteins. We detected a remarkable alteration in the anaphase distribution of Aurora B and INCENP, which are key regulators of chromosome segregation. In particular, a fraction of both proteins remained abnormally loaded on chromosomes during anaphase in **MCPH** patients' cells while in cells of normal control subjects both proteins are completely transferred to the spindle midzone. We did not observe any other alterations regarding cell cycle progression, chromosome structure, or response to DNA damage. Our observations point towards a molecular role of the underlying gene product in the regulation of anaphase/telophase progression possibly through interaction with chromosomal passenger proteins. In addition, our findings represent further evidence for the proposed role of **MCPH** genes in the regulation of mitotic progression.

PMID: 21857152 [PubMed - indexed for MEDLINE] **Free full text**

Publication Types, MeSH Terms, Substances

LinkOut - more resources

Mol Syndromol. 2012 Jun;3(1):6-13. Epub 2012 Jun 13.

Two Missense Mutations in the Primary Autosomal Recessive Microcephaly Gene MCPH1 Disrupt the Function of the Highly Conserved N-Terminal BRCT Domain of Microcephalin.

Ghani-Kakhki M, Robinson PN, Morlot S, Mitter D, Trimborn M, Albrecht B, Varon R, Sperling K, Neitzel H.

Institute of Medical and Human Genetics, Charité - Universitätsmedizin Berlin, Berlin, Germany.

Abstract

Primary microcephaly **MCPH1** is an extremely rare autosomal recessive disorder associated with congenital microcephaly, mental retardation and a distinctive cellular phenotype of misregulated chromosome condensation. The **MCPH1** gene encodes an 835-amino acid protein, microcephalin, which contains 1 N-terminal and 2 C-terminal BRCT (BRCA1 C-terminus) domains. BRCT domains are predominantly found in proteins involved in cell cycle control and DNA repair. Here we describe 1 novel and 1 previously reported **MCPH1** missense mutation, p.Trp75Arg and p.Ser72Leu, respectively, in the N-terminal BRCT domain of microcephalin associated with severe congenital microcephaly. Both residues are entirely conserved in the **MCPH1** orthologs of all vertebrate species and Drosophila. Proliferating lymphocytes of the patients with p.Trp75Arg and p.Ser72Leu show the unique cellular **MCPH1** phenotype of misregulated chromosome condensation, indicating that these missense alterations disrupt the function of the N-terminal BRCT domain of the protein. Interestingly, both residues are strictly conserved in BRCT domains of BRCA1. ClustalW alignments show that the residue p.Ser72 of microcephalin corresponds to p.Ser1715 of the N-terminal BRCT domain of BRCA1, while the microcephalin residue p.Trp75 is analogous to p.Trp1718 in the N-terminal BRCT and to p.Trp1837 in C-terminal BRCT domains of BRCA1. Missense alterations for all 3 corresponding BRCA1 residues were described and are predicted to be deleterious resulting in the destabilization of the BRCA1 protein. Our data on the 2 **MCPH1** missense alterations provide further evidence for the functional significance of these residues in BRCT domains.

PMID: 22855649 [PubMed] PMCID: PMC3398827 **Free PMC Article**

J Med Genet. 2010 Dec;47(12):823-8. doi: 10.1136/jmg.2009.076398. Epub 2010 Oct 26.

A clinical and molecular genetic study of 112 Iranian families with primary microcephaly.

Darvish H, Esmaeeli-Nieh S, Monajemi GB, Mohseni M, Ghasemi-Firouzabadi S, Abedini SS, Bahman I, Jamali P, Azimi S, Mojahedi F, Dehghan A, Shafeghati Y, Jankhah A, Falah M, Soltani Banavandi MJ, Ghani-Kakhi M, Garshasbi M, Rakhshani F, Naghavi A, Tzschach A, Neitzel H, Ropers HH, Kuss AW, Behjati F, Kahrizi K, Najmabadi H.

Genetics Research Centre, University of Social Welfare and Rehabilitation Sciences, Kodakyar Street, Daneshjo Ave, Tehran, Iran.

Abstract

BACKGROUND: Primary microcephaly (MCPH) is a genetically heterogeneous disorder showing an autosomal recessive mode of inheritance. Affected individuals present with head circumferences more than three SDs below the age- and sex-matched population mean, associated with mild to severe mental retardation. Five genes (**MCPH1**, CDK5RAP2, ASPM, CENPJ, STIL) and two genomic loci, MCPH2 and MCPH4, have been identified so far.

METHODS AND RESULTS: In this study, we investigated all seven MCPH loci in patients with primary microcephaly from 112 Consanguineous Iranian families. In addition to a thorough clinical characterisation, karyotype analyses were performed for all patients. For Homozygosity mapping, microsatellite markers were selected for each locus and used for genotyping. Our investigation enabled us to detect homozygosity at **MCPH1** (Microcephalin) in eight families, at MCPH5 (ASPM) in thirteen families. Three families showed homozygosity at MCPH2 and five at MCPH6 (CENPJ), and two families were linked to MCPH7 (STIL). The remaining 81 families were not linked to any of the seven known loci. Subsequent sequencing revealed eight, 10 and one novel mutations in Microcephalin, ASPM and CENPJ, respectively. In some families, additional features such as short stature, seizures or congenital hearing loss were observed in the microcephalic patient, which widens the spectrum of clinical manifestations of mutations in known microcephaly genes.

CONCLUSION: Our results show that the molecular basis of microcephaly is heterogeneous; thus, the Iranian population may provide a unique source for the identification of further genes underlying this disorder.

PMID: 20978018 [PubMed - indexed for MEDLINE]

Publication Types, MeSH Terms, Substances

LinkOut - more resources

Statement of Authorship

I, Mahdi Ghani-Kakhki, declare that I made the following contributions to the selected publications

Publication 1

Trimborn M, Ghani M, Walther DJ, Dopatka M, Dutrannoy V, Busche A, Meyer F, Nowak S, Nowak J, Zabel C, Klose J, Esquitino V, Garshasbi M, Kuss AW, Ropers HH, Mueller S, Poehlmann C, Gavvovidis I, Schindler D, Sperling K, Neitzel H. Establishment of a Mouse Model with Misregulated Chromosome Condensation due to Defective Mcph1 Function. PLoS One. 2010 Feb 16;5(2):e9242.

Proportion ~ 50%

This paper is related to the main part of my PhD project; the identification of the gene trap insertion site by me was a critical finding. In addition; the multiple fibroblast cell lines that were established and studied for their cellular phenotype by me confirmed that the mice showed the PCC phenotype. The most significant results were that mice cells displayed the PCC phenotype, despite lacking only the C-terminal domain, as early as one hour after S-phase. Moreover, I also performed other complementary experiments such as long range PCR, RT-PCRs, DNA extractions and mice genotyping.

Publication 2

Marchal JA, Ghani M, Schindler D, Gavvovidis I, Winkler T, Esquitino V, Sternberg N, Busche A, Krawitz P, Hecht J, Robinson P, Mundlos S, Graul-Neumann L, Sperling K, Trimborn M, Neitzel H. Misregulation of mitotic chromosome segregation in a new type of autosomal recessive primary microcephaly. Cell Cycle 10: 2967-77, 2011.

Proportion ~ 35%

Concerning the identification of the new locus, I was involved in the microsatellite genotyping to exclude the known loci, sequencing of some candidate genes, and in the genetic mapping of the new locus.

Publication 3

Ghani M, Robinson P, Morlot S, Mitter D, Trimborn M, Albrecht B, Varon R, Sperling K, Neitzel H. Two new missense mutations in primary autosomal recessive microcephaly MCPH1 disrupt the function of the highly conserved N-terminal BRCT domain of microcephalin. (Molecular Syndromology, 2012;3:6-13)

Proportion ~ 70%

The results are mainly based on the molecular genetic, cytogenetic and bioinformatic analyses I did.

Publication 4

Darvish H, Esmaeeli-Nieh S, Monajemi GB, Mohseni M, Ghasemi-Firouzabadi S, Abedini SS, Bahman I, Jamali P, Azimi S, Mojahedi F, Dehghan A, Shafeghati Y, Jankhah A, Falah M, Soltani Banavandi MJ, Ghani-Kakhi M, Garshasbi M, Rakhshani F, Naghavi A, Tzschach A, Neitzel H, Ropers HH, Kuss AW, Behjati F, Kahrizi K, Najmabadi H. A clinical and molecular genetic study of 112 Iranian families with primary microcephaly. J Med Genet. 47::823-8, 2010

Proportion ~ 15%

The paper reports on 19 different mutations including the three that I had found. I could identify there the first missense variation of the *ASPM* gene.

Declaration

I, Mahdi Ghani-Kakhki, declare that the dissertation on "Molecular genetic and cytogenetic analyses of autosomal recessive primary microcephaly (MCPH); Mouse model, new locus and novel mutations"has been written by myself without the help of (unauthorised) third parties, that neither the work or parts thereof are copies of other works and that all aids and literature used are listed in their entirety.

April 04, 2012 Signature

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The project would not be successful without MCPH families who agreed to participate in this project and I am so indebted to all of them. I also appreciate the support of all physicians and nurses who helped in this study.

Last but not least, I would like to acknowledge and recognize the kindness, patience and support from my family members, my wife Fahimeh, my son Erfan and my newborn baby Ava during this PhD dissertation. I am also thankful to both my parents for insisting that I pursue my education.

Curriculum Vitae (CV)

My CV will not be published in the electronic version of my work for privacy reasons.

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.