Chapter 6

GPSM2 and Chudley–McCullough Syndrome: A Dutch Founder Variant Brought to North America

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Abstract

Chudley–McCullough syndrome (CMS) is characterized by profound sensorineural hearing loss and brain anomalies. Variants in GPSM2 have recently been reported as a cause of CMS by Doherty et al. In this study we have performed exome sequencing of three CMS patients from two unrelated families from the same Dutch village. We identified one homozygous frameshift GPSM2 variants c.1473delG in all patients. We show that this variant arises from a shared, rare haplotype. Since the c.1473delG variant was found in Mennonite settlers, it likely originated in Europe. To support DNA diagnostics, we established an LOVD database for GPSM2 containing all variants thus far described.

Introduction

Chudley–McCullough syndrome (CMS) is characterized by profound congenital sensorineural hearing loss associated with (partial) agenesis of the corpus callosum, colpocephaly (enlargement of the occipital horns), hydrocephaly, and other brain abnormalities such as arachnoid cysts, gray matter hetero- topia, and cortical dysplasia [Ostergaard et al., 2004]. This syndrome was first recognized in a brother and sister by Chudley et al. [1997]. Based on affected sibs of both sexes from phenotypically normal parents the syndrome was assumed to be an autosomal recessive trait. Subsequent reports have supported this assumption, describing parental consanguinity or origin from a small community [Chudley et al., 1997].

Patients with CMS may either be hearing or deaf at birth. However, hearing loss is always profound by the age of 3 years [Hendriks et al., 1999; Lemire and Stoeber, 2000; Welch et al., 2003; Ostergaard et al., 2004; Matteucci et al., 2006]. It is has been suggested that some cases of CMS may not be detected because the hydrocephalus does not progress and is compensated [Welch et al., 2003].

Inactivating mutations in the GPSM2 gene have been linked to both autosomal recessive non-syndromic (DFNB82) and syndromic hearing loss. Doherty et al. [2012] recently linked inactivating mutations in GPSM2 to CMS. They successfully applied exome sequencing in conjunction with homozygosity mapping to identify four deleterious mutations (c.1473delG (c.1471delG in Doherty et al.), c.742delC (c.741delC in Doherty et al.), c.1661C>A and

c.1062þ1G>T) in affected individuals with CMS from eight fam- ilies. In this study, two other families with CMS were investigated; the same c.1473delG variant in three patients from two unrelated Dutch families was identified. Together, the c.742delC and c.1473delG founder mutations seem to be a frequent cause of CMS, as they are observed in homozygous form in 8/10 families reported thus far, and in heterozygous form in an additional family. In the present study we confirm that mutations in GPSM2 gene are responsible for CMS and show that at least a part of the CMS cases are due to a founder effect.

Materials and Methods

Patients

In our clinic we had three CMS patients in two different families (Fig. 1). In the first family, described previously by Hendriks et al. [1999], two affected sisters had a combination of congenital sensorineural hearing loss, partial agenesis of the corpus callosum, arachnoid cyst, and hydrocephalus. They had normal development and no distinctive physical anomalies. Their parents were non- consanguineous but originated from the same Dutch village, were phenotypically normal and both had normal hearing and no brain abnormalities. Hendriks et al. postulated that the two affected sibs may have had a different syndrome than that described by Chudley et al. Welch et al. later commented that the two affected girls most likely had CMS [Hendriks et al., 1999; Welch et al., 2003]. Recently the two sisters were re-examined at the age of 17 and 25 years, respectively, and had normal intelligence.

The second family included a single affected patient who was born after an uneventful pregnancy. A structural ultrasound study at a gestational age of 20 weeks did not show abnormalities. During pelvic examination at labor a hydrocephalus was suspected and a subsequent ultrasound revealed ventriculomegaly and cesarean section was performed. The patient was born at term. Postnatal brain imaging (MRI) revealed colpocephaly, agenesis of the corpus callosum, heterotopia, an interhemispheric cyst at the dorsum of the third ventricle, polymicrogyria of frontal lobes and cerebellar dysgenesis. The patient was also diagnosed with severe sensorineu- ral hearing loss (no response at 90 dB). She had a normal develop- ment at the age of 2 years except for her delay in speech development. After she received a cochlear implant at the age of 2 years and 4 months, there was an improvement in hearing (30 –40 dB hearing

level) and in active vocabulary. The family historywas unremarkable. Physical findings were normal (head circum- ference: +2 SDS).

Metabolic and DNA analysis of several genes related to hearing loss (GJB2, GJB6, SLC26A4 gene) or brain anomalies (GPR56 gene) did not result in an obvious cause for the malformations. Based on the presence of these rather specific clinical findings, the patient was diagnosed as having CMS.

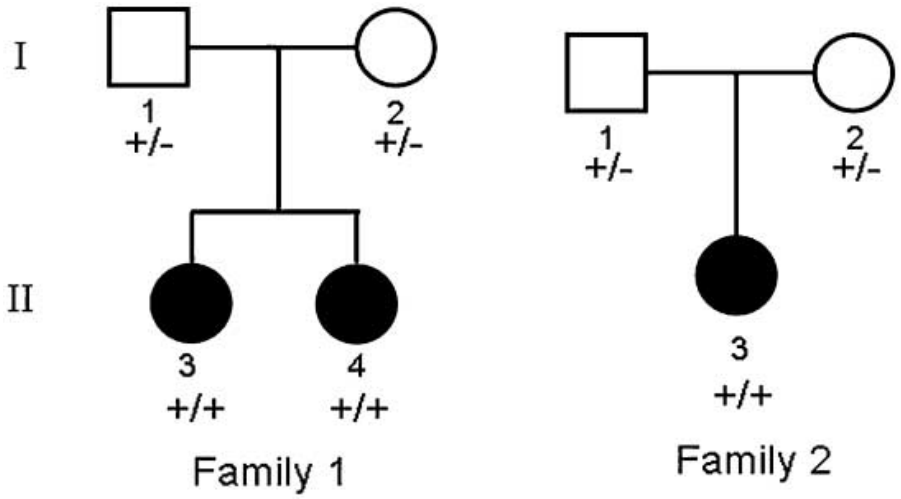

Figure 1. Pedigrees of the two families with Chudley-McCullough syndrome. Black symbols: affected patients. +/+: homozygous for the c.1473delG variant; +/-: heterozygous for the c.1473delG variant.

Exome Sequencing

As the gene for CMS was not yet identified at the time of this investigation, we have applied exome sequencing to resolve the genetic basis of CMS in these two families. We sequenced the exomes of the sibs from family one and the sporadic patient from family two. Whole exome capture was performed using Agilent's 50 Mb Sure- select exome capture kit following instructions provided by the manufacturers (SureSelect, Agilent). In brief, 3 mg of DNA was fragmented (Covaris) to yield fragments of 300–400 bp. Paired- end adaptors with index from Illumina were added to both ends. The DNA-adaptor-ligated fragments were then hybridized to 250 ng of SureSelect whole exome probes capture library (SureSelect, Agilent) for 30h. After capture, a qPCR assay was done to calculate the relative fold-enrichment prior for sequencing. The eluted-enriched DNA fragments were sequenced using the Hiseq 2000 platform (Illumina). BWA [Li and Durbin, 2009] was used to map the data to human genome build 37 (hg19), and

GATK tools [McKenna et al., 2010] were used to perform the data analysis with minor modifications described elsewhere [Santen et al., 2012].

Sanger Sequencing

PCR was done by using Phire Hot Start II DNA polymerase following the official protocol. Primers used in PCR reactions are available upon request. After PCR, fragments were first purified by QIAquick PCR purification kit (Qiagen), then mixed with 10 pmol of the forward or reverse primers and sequenced by the Applied Biosystems 96-capillary (3730XL system).

Results

Using the GATK sequence analysis pipeline, we identified 26,487 possibly shared variants in the two siblings and 22,901 variants in the sporadic patient located in the exons and exon/intron junctions. After filtering to exclude all known variants in databases (dbSNP135, 1000 Genomes Project, and the in-house database) 278 and 181 variants remained, respectively. Furthermore, filtering for recessive inheritance left only a homozygous single base pair deletion in *GPSM2* which causes a frameshift and a premature stop (NM_013296.4:c.1473delG, p.Phe492SerfsX5). This variant was confirmed in all patients and in heterozygous form in their parents by Sanger sequencing.

Since both of our families came from the same small Dutch village and shared the same homozygous variant, we used the exome data to reveal a possible link between the two families. We found two other rare homozygous variants shared by all three patients, both of which within 0.5 Mbp of the *GPSM2* variant (g.109477462G>C; NM_015127.3(CLCC1):c.1336C>G; p.Pro446Ala and g.109909853A>C; NM_002959.5(SORT1):c.440+177T>G). To further define the size of the haplotype, we looked at high quality calls of known SNPs in this genomic region and found that the region of homozygous SNPs shared by all three patients is flanked by rs6672483 and rs333967 (Fig. 2), indicating that the maximal size of the shared haplotype is 2.2 Mb. The shared genotype for the two sisters spans 14.7 Mb (Fig. 2). The sporadic patient has a homozygous stretch of 19 Mb (Fig. 2). None of the three rare variants shared by the three patients was identified in the Genome of the Netherlands (consisting of 250 completely sequenced trios, 500 independent genomes, www.nlgenome.nl). The g.109477462G>C variant

was found in 0.2% of the subjects with European ancestry sequenced in the National Heart, Lung, and Blood Institute Grand Opportunity Exome Sequencing Project (NHLBI GO ESP).

We further investigated whether the distant relation of the three patients was reflected by the mitochondrial DNA (i.e. the maternal line). We found 19 positions with variants on the mitochondrial DNA covered at least 10x in all of the subjects. There was a 100% concordance between the two sisters and only one position in which the sporadic patient differed. We compared the concordance with three other Dutch patients from different projects and found a much lower concordance in those patients (44-55%).

To support DNA diagnostic studies we established a Leiden Open Variation Database (LOVD) for *GPSM2* containing all variants that have been published thus far (www.LOVD.nl/GPSM2).

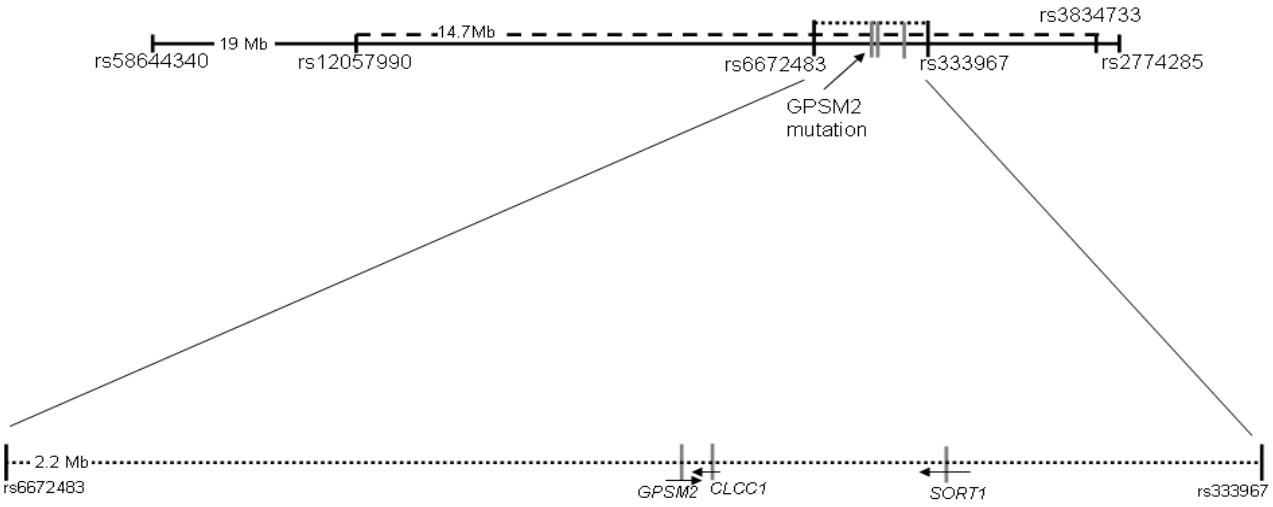

Figure 2. Representation of the haplotype information around the *GPSM2* mutation. The three vertical gray lines represent the rare homozygous variants shared by all three patients in *GPSM2, CLCC1* and *SORT1*, respectively. The dotted line represents the homozygous stretch shared by all three patients (2.2 Mb). The striped line represents the stretch where the two sisters share the same genotype (14.7 Mb). The continuous line represents the homozygous stretch for the sporadic patient (19 Mb).

Discussion

GPSM2 was linked to autosomal recessive non-syndromic hearing loss DFNB82 by Walsh et al, who described the successful application of exome sequencing in conjunction with homozygosity mapping to identify a nonsense variant in *GPSM2* (c.379C>T;p.Arg127*) [Walsh et al., 2010]. Subsequently, Yariz et al. [2012] reported a second truncating variant in *GPSM2*

(c.1684C>T;p.Q562*) by autozygosity mapping followed by candidate gene analysis in a consanguineous Turkish family with nonsyndromic hearing loss. While preparing this manuscript, Doherty et al. [2012] reported the identification of variants in *GPSM2* as a cause of CMS. They identified the c.1473delG variant in patients from Mennonite ancestry (reported as c.1471delG) [Doherty et al., 2012]. The authors hypothesize that it is from European origin, which we can now confirm. The size we computed for the common haplotype was 2.2 Mb, which is smaller but in the same order of magnitude as the 2.9 Mb haplotype observed in the Mennonite families [Doherty et al., 2012]. Since none of the variants was present in the database of the Genome of The Netherlands, and one of the variants was present in a low frequency in the Exome Variant Server (NHLBI GO ESP), we conclude that this haplotype is very rare, and represents a founder haplotype in the village of origin. This is further strengthened by the mitochondrial data, which shows that the two families are related in the maternal line, and by the fact that we have identified another unrelated family with the same homozygous mutation from this village (data not shown).

We have created a variant database (www.LOVD.nl/GPSM2) for *GPSM2*. The value of this database is in enhanced interpretation for diagnostic use, but also facilitates comparison between studies. The c.1473delG variant that we identified was erroneously annotated as c.1471delG in Doherty et al., 2012. The creation of a database which checks for HGVS nomenclature partly resolves such differences between papers.

GPSM2 (the G protein signaling modulator 2) also known as *LGN* and *Pins,* contains 14 exons, and spans 55,073 bp on chromosome 1p13.3. It encodes a 684 amino acid protein. *GPSM2* has 6 transcripts according to the Ensembl database, ranging in size from 571 to 3310 bp. The GPSM2 protein is widely expressed [Blumer et al., 2002]. However, highest expression is seen during embryonic development. Its functional role relates to cell polarity and spindle orientation, for example, in cells of the developing cerebral cortex in mice [discussed by Doherty et al., 2012]. The protein is comprised of seven N-terminal tetratricopeptide (TPR) motifs, a linker domain, and four C-terminal (GoLoco) motifs which are involved in guanine nucleotide exchange [Du et al., 2001; Johnston et al., 2009; Willard et al., 2008]. The 1 bp deletion in exon 13 locates within the C-terminal GoLoco motif and creates a frame shift starting at codon Phe492 and ends in a stop codon four positions downstream, leading to a functional absence of the GPSM2 protein.

Our results confirm that inactivating mutations in *GPSM2* cause Chudley McCollough syndrome. The c.1473delG mutation in *GPSM2* associated with CMS appears to be an ancient founder mutation brought to North America by Mennonite settlers originating from Western Europe. Together, the c.742delC and c.1473delG founder mutations seem to be a frequent cause of CMS. Future work will need to show if an ascertainment bias has inflated the importance of these mutations.

Acknowledgments

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Web Resources

GPSM2, Leiden Open Variant Database: http://www.lovd.nl/gpsm2

Genome of the Netherlands: http://www.nlgenome.com

NHLBI GO ESP: http://evs.gs.washington.edu/EVS/

References

Blumer JB, Chandler LJ, Lanier SM. 2002. Expression analysis and subcellular distribution of the two G-protein regulators AGS3 and LGN indicate distinct functionality. Localization of LGN to the midbody during cytokinesis. J Biol Chem 277:15897–15903.

Chudley AE, McCullough C, McCullough DW. 1997. Bilateral sensorineural deafness and hydrocephalus due to foramen of Monro obstruction in sibs: a newly described autosomal recessive disorder. Am J Med Genet 68:350-6.

Doherty D, Chudley AE, Coghlan G, Ishak GE, Innes AM, Lemire EG, Rogers RC, Mhanni AA, Phelps IG, Jones SJ, Zhan SH, Fejes AP, Shahin H, Kanaan M, Akay H, Tekin M; FORGE Canada Consortium, Triggs-Raine B, Zelinski T. 2012. *GPSM2* mutations cause the brain malformations and hearing loss in Chudley-McCullough syndrome. Am J Hum Genet 90:1088-93.

Du Q, Stukenberg PT, Macara IG. 2001. A mammalian partner of inscrutable binds NuMA and regulates mitotic spindle organization. Nat Cell Biol 3:1069–1075.

Johnston CA, Hirono K, Prehoda KE, Doe CQ. 2009. Identification of an Aurora-A/PinsLINKER/Dlg spindle orientation pathway using induced cell polarity in S2 cells. Cell 138: 1150–1163.

Hendriks YMC, Laan LAEM, Vielvoye GJ, Van Haeringen A. 1999. Bilateral sensorineural deafness, partial agenesis of the corpus callosum, and arachnoid cysts in two sisters. Am J Med Genet 86:183–186.

Lemire EG, Stoeber GP. 2000. Chudley-McCullough syndrome: bilateral sensorineural deafness, hydrocephalus, and other structural brain abnormalities. Am J Med Genet 90:127–130.

Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754-1760.

Matteucci F, Tarantino E, Bianchi MC, Cingolani C, Fattori B, Nacci A, Ursino F. 2006. Sensorineural deafness, hydrocephalus and structural brain abnormalitiesin two sisters: the Chudley-McCullough syndrome. Am J Med Genet 140:1183–1188.

McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. 2010. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 20:1297-1303.

Ostergaard E, Pedersen VF, Skriver EB, Brøndum-Nielsen K. 2004. Brothers with Chudley-McCullough syndrome: sensorineural deafness, agenesis of the corpus callosum, and other structural brain abnormalities. Am J Med Genet 124:74-8.

Santen GW, Aten E, Sun Y, Almomani R, Gilissen C, Nielsen M, Kant SG, Snoeck IN, Peeters EA, Hilhorst-Hofstee Y, Wessels MW, den Hollander NS, Ruivenkamp CA, van Ommen GJ, Breuning MH, den Dunnen JT, van Haeringen A, Kriek M. 2012. Mutations in SWI/SNF chromatin remodeling complex gene *ARID1B* cause Coffin-Siris syndrome. Nat Genet 44:379-80

Walsh T, Shahin H, Elkan-Miller T, Lee MK, Thornton AM, Roeb W, Abu Rayyan A, Loulus S, Avraham KB, King MC, Kanaan M. 2010. Whole exome sequencing and homozygosity mapping identify mutation in the cell polarity protein GPSM2 as the cause of nonsyndromic hearing loss DFNB82. Am J Hum Genet 87:90-4.

Welch KO, Tekin M, Nance WE, Blanton SH, Arnos KS, Pandya A. 2003. Chudley-McCullough syndrome: expanded phenotype and review of the literature. Am J Med Genet 119:71–76.

Willard FS, Zheng Z, Guo J, Digby GJ, Kimple AJ, Conley JM, Johnston CA, Bosch D, Willard MD, Watts VJ, Lambert NA, Ikeda SR, Du Q, Siderovski DP. 2008. A point mutation to Galphai selectively blocks GoLoco motif binding: Direct evidence for Galpha.GoLoco complexes in mitotic spindle dynamics. J Biol Chem 283:36698–36710.

Yariz KO, Walsh T, Akay H, Duman D, Akkaynak AC, King MC, Tekin M. 2012. A truncating mutation in GPSM2 is associated with recessive non-syndromic hearing loss. Clin Genet 81:289-93.