

Genotype and Phenotype Investigations in Camptodactyly-Arthropathy-Coxa-Vara-Pericarditis (CACP) Syndrome

Saliha Yilmaz^{1,2*}

¹Department of Neurosurgery and Genetics, Yale Program in Brain Tumor Research, Yale School of Medicine, New Haven, CT 06510, USA

²Department of Research Genetics, WuXi Nextcode Genomics, 8th Floor, 55 Cambridge Pkwy, Cambridge, MA 02142, USA.

Received July 13, 2018; Accepted August 30, 2018; Published November 28, 2018

INTRODUCTION

The camptodactyly–arthropathy–coxa–vara–pericarditis syndrome (CACP) is a rare autosomal recessive condition characterized by early onset camptodactyly, noninflammatory arthropathy with synovial hyperplasia, and progressive coxa vara deformity (MIM # 208250). The syndrome was first reported in the late seventies [1,2] and it is in 1999 that Proteoglycan 4 gene (PRG4; OMIM: 604283) was implicated in the disease [3]. To this date, only 8 genetics studies on CACP were published in the scientific literature. Our recent work on 11 unrelated families is one of the largest CACP cohort analyzed [4]. With this mini-review, we aim to recapitulate the genetic and phenotypic findings from our recent paper as well as available scientific literature to date.

PATIENT PHENOTYPES AND FAMILY CHARACTERISTICS

Demographic

At the time of this review, most of the published cases are from regions or countries where the consanguinity rate is high. Indeed, CACP being a rare and recessive disease it is more prevalent in inbred families. For example, ten of the eleven families presented in our study were from southeast region of Turkey where rates of consanguineous marriage are high, and nine of families are reported to be consanguineous. Likewise, most of the previous published cases are from countries such as Saudi Arabia, United Arab Emirates, Egypt and Pakistan where consanguinity rates are high [4-7].

Gender difference

Several reports have observed an over-representation of male CACP patients. However, there is not enough evidence to conclude with the number of samples available to date [4].

Symptoms and (mis)-diagnosis

Camptodactyly of the hands represents the earliest symptom appearing during the first weeks or months of life, while the

wrists represent the first large joints affected in early childhood period [4,6,8-10]. The report of coxa vara varies with studies reporting figures between 50% and 90% of CACP patients. All of patients in our study presented coxa vara and broad and short femoral neck as most distinct radiological findings. Two patients from family 2 and one patient from family 3 had pericarditis. In addition, four patients had MVP and MR on echocardiography consistent with the numbers reports by others [11].

CACP is a rare disease with symptoms common to Rheumatologic disorders. Indeed, sixteen of our patients were referred for genetic evaluation with the initial diagnosis of JIA and only 17% (6 of 35) of our patients were diagnosed before 5 years of age. The misdiagnose and unnecessary treatment with anti-inflammatory drugs has been frequently noticed among authors [11,12].

GENOTYPE AND PHENOTYPE ANALYSES

PRG4 mutation landscape

Our genomic and molecular screening identified 6 frame shift mutations, 2 nonsense mutations, and the 1st case of homozygous deletion of exon 1. Among the 27 mutations reported in the literature since 1993 [3], 15 are frame shift mutations, 4 stop codons, and 1 splice site acceptor. Our study brings the total number of disease-causing mutations from 25 to 38. The higher number of mutations observed on exon 6 does not seem to represent a mutation hotspot with the number of cases published so far

Corresponding author: Saliha Yilmaz, Department of Research Genetics, WuXi Nextcode Genomics, 8th Floor, 55 Cambridge Pkwy, Cambridge, MA 02142, USA, E-mail: syilmaz@wuxinextcode.com; yilmaz.saliha@gmail.com

Citation: Yilmaz S. (2018) Genotype and Phenotype Investigations in Camptodactyly-Arthropathy-Coxa-Vara-Pericarditis(CACP) Syndrome. J Genomic Med Pharmacogenomics, 3(1): 338-341.

Copyright: © 2018 Yilmaz S. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

(binominal test $p=0.86$). However, there seems to be a significantly higher number of mutations in the regions not involved in alternative splicing (binominal test $p=0.046$). This would support the idea that CACP appears when there is not any functional PRG4 protein left. Finally, only 2 mutations among the 38 have been described as escaping the nonsense-mediated mRNA decay (NMD) system [3,4].

Dysfunctional or absent PRG4 protein?

Based on CACP mutation profiles, several authors previously stipulated that the syndrome is due to a complete lack of the protein PRG4 [6,8,13]. In addition, studies using an antibody against the C-terminal and N-terminal of PRG4 protein showed its absence in CACP patient's synovial fluid, while it was detected in sample patients with rheumatoid arthritis and osteoarthritis [14]. It has also been shown that the synovial fluid from patients with CACP lack lubricating properties [15].

Also, one CACP family with a dinucleotide transversion (4190CC→AG) creating a nonsense codon on the last exon has been reported [3]. *In vitro* experiments have shown that this mutated protein, which is not degraded by NMD [16,17] does not undergo the normal process of SPC-mediated cleavage within the PEX domain [18]. This would mean that PRG4 protein is nonfunctional but present as a truncated form [19-21]. In our cohort, we report three siblings with homozygous p.Y1367X (c.4101C>G) mutation predicted to escape the NMD. We did not observe any significant phenotypic difference compared to the rest of the families with deleterious mutations predicted to activate NMD [4].

CACP has been described as a clinically variable but genetically homogenous disease [4,9] and the disease inter- and intra-familial variability had been repetitively described by authors, without any emerging consensus on the origins of such variability.

CACP disease progress with time

We confirmed a significant correlation between the age of the patient and the number of clinical features [4]. This increase in symptoms is likely due to cumulative mechanical stress over time [22,23]. Differences in mechanical stress between individuals may explain part of the intrafamilial variability. Indeed, we observe that for family 1, the accumulation of skeletal features was proportional to the age of the patient. For other families there was more variability in Phenoscore (a score counting the number of skeletal symptoms), even though a positive correlation existed.

Location and nature of the PRG4 mutations

Even though we observe a trend in a majority of our cohort, the first reported symptoms among individuals remain variable. This is true for individuals within the same family as well as between families [4,6,8-10]. Moreover, for a similar age, the nature and severity of skeletal features are also variable between patients. For example, for the same

age, the skeletal findings were more numerous, but milder in family 2 compared to family 1. However, we did not find any correlation between the location or the type of the mutation and the outcome.

Non-sense mediated decay system (NMD)

It has been reported that the NMD efficiency is variable between individuals and that difference of efficiency could explain some interindividual variabilities in phenotypes [24]. Studies in mice have supported this hypothesis [25]. Importantly, premature termination codons (PTCs) that are unable to trigger NMD can cause dominantly inherited forms of the disease, as for example, β -thalassemia [26,27]. Besides the first methionine codon, PRG4 contains Met55 on exon 2, 13 methionine codons on exon 6, and 1 in exon 8. Unfortunately, it is currently not possible to predict which mRNA will trigger or escape NMD based on the sequence features only [28]. Future studies aiming to study (NMD) system efficiently on CACP patients' samples could help define the importance of this mechanism in CACP patients' phenotype variability.

Modifier genes

We observed several extra clinical features present in our CACP patients. Approximately 20% of patients with CACP also had pericarditis, which was not associated with age, gender, or mutation type or localization. This prone the idea of modifier genes. Indeed, the presence of additional variants (common or rare; ubiquitous or tissue specific) could fine tune the traits produced by the malfunction of PRG4 protein or account for the extra skeletal feature present in a minority of CACP patients. Indeed, 80% of the families are consanguineous, and the probability of accumulating homozygous genomic mutations is therefore higher than in the general population. Modern genomic studies have shown that "seemingly" monogenic diseases phenotype is fine-tuned by modifier genes [29,30]. For example, cystic fibrosis is an example of an autosomal recessive disease showing a very complex association between genotype and clinical phenotype. Indeed, it is not possible to predict individual outcome based on cystic fibrosis transmembrane regulator gene (CFTR; OMIM: 602421) genotype only. The expression of the disease is influenced by various factors that make phenotype variability extend along a wide spectrum [28]. Among the patients in our cohort with exome sequencing information, we searched for co-concurrent mutations in genes that may be linked to PRG4 or its pathways (hyaluronan synthase 1: HAS1; OMIM: 601463; aggrecan: ACAN; OMIM: 155760). With limited number of data and samples we present here, it is not possible to draw any conclusion about secondary mutations and the analyses in our 2018 paper were purely exploratory by nature.

CONCLUSION

CACP is a disorder that effects large and small joints, progress with the age of the patient and shows intra- and interfamilial clinical variations. The interfamilial variabilities as well as CACP's nonskeletal features do not seem to correlate with age, gender, ethnicity, and geographic localization. CACP appears when both copies of PRG4 are dysfunctional. However, the total absence of PRG4 protein might not require leading to the disease. Larger cohorts with extensive clinical data and exome sequencing methods could elucidate the wide clinical variability.

REFERENCES

- Athreya BH, Schumacher HR (1978) Pathologic features of a familial arthropathy associated with congenital flexion contractures of fingers. *Arthritis Rheum* 21: 429-437.
- Jacobs JC, Phillips PE, Johnston AD (1976) Needle biopsy of the synovium of children. *Pediatrics* 57: 696-701.
- Marcelino J, Carpten JD, Suwairi WM, Gutierrez OM, Schwartz S, et al. (1999) CACP, encoding a secreted proteoglycan, is mutated in camptodactyly-arthropathy-coxa vara-pericarditis syndrome. *Nat Genet* 23: 319-322.
- Yilmaz S, Uludag Alkaya D, Kasapcopur O, Barut K, et al. (2018) Genotype-phenotype investigation of 35 patients from 11 unrelated families with camptodactyly-arthropathy-coxa vara-pericarditis (CACP) syndrome. *Mol Genet Genomic Med* 6: 230-248.
- Akawi NA, Ali BR, Al-Gazali L (2012) A novel mutation in PRG4 gene underlying camptodactyly-arthropathy-coxa vara-pericarditis syndrome with the possible expansion of the phenotype to include congenital cataract. *Birth Defects Res A Clin Mol Teratol* 94: 553-556.
- Alazami AM, Al-Mayouf SM, Wyngaard CA, Meyer B (2006) Novel PRG4 mutations underlie CACP in Saudi families. *Hum Mutat* 27: 213.
- Albuhairan I, Al-Mayouf SM (2013) Camptodactyly-arthropathy-coxavara-pericarditis syndrome in Saudi Arabia: Clinical and molecular genetic findings in 22 patients. *Semin Arthritis Rheum* 43: 292-296.
- Basit S, Iqbal Z, Umicevic-Mirkov M, Kamran Ul-Hassan Naqvi S, Coenen M, et al. (2011) A novel deletion mutation in proteoglycan-4 underlies camptodactyly-arthropathy-coxa-vara-pericarditis syndrome in a consanguineous pakistani family. *Arch Med Res* 42: 110-114.
- Faivre L, Prieur AM, Le Merrer M, Hayem F, Penet C, et al. (2000) Clinical variability and genetic homogeneity of the camptodactyly-arthropathy-coxa vara-pericarditis syndrome. *Am J Med Genet* 95: 233-236.
- Offiah AC, Woo P, Prieur AM, Hasson N, Hall CM (2005) Camptodactyly-arthropathy-coxa vara-pericarditis syndrome versus juvenile idiopathic arthropathy. *AJR Am J Roentgenol* 185: 522-529.
- Nandagopalan RS, Phadke SR, Dalal AB, Ranganath P (2014) Novel mutations in PRG4 gene in two Indian families with camptodactyly-arthropathy-coxa vara-pericarditis (CACP) syndrome. *Indian J Med Res* 140: 221-226.
- Bulutlar G, Yazici H, Ozdogan H, Schreuder I (1986) A familial syndrome of pericarditis, arthritis, camptodactyly and coxa vara. *Arthritis Rheum* 29: 436-438.
- Ciullini Mannurita S, Vignoli M, Bianchi L, Kondi A, et al. (2014) CACP syndrome: Identification of five novel mutations and of the first case of UPD in the largest European cohort. *Eur J Hum Genet* 22: 197-201.
- Ai M, Cui Y, Sy MS, Lee DM, Zhang LX, et al. (2015) Anti-lubricin monoclonal antibodies created using lubricin-knockout mice immunodetect lubricin in several species and in patients with healthy and diseased joints. *PLoS One* 10: e0116237.
- Jay GD, Torres JR, Rhee DK, Helminen HJ, Hytinen MM, et al. (2007) Association between friction and wear in diarthrodial joints lacking lubricin. *Arthritis Rheum* 56: 3662-3669.
- Brogna S, Wen J (2009) Nonsense-mediated mRNA decay (NMD) mechanisms. *Nat Struct Mol Biol* 16: 107-113.
- Perrin-Vidoz L, Sinilnikova OM, Stoppa-Lyonnet D, Lenoir GM, Mazoyer S (2002) The nonsense-mediated mRNA decay pathway triggers degradation of most BRCA1 mRNAs bearing premature termination codons. *Hum Mol Genet* 11: 2805-2814.
- Rhee DK, Marcelino J, Al-Mayouf S, Schelling DK, Bartels CF, et al. (2005) Consequences of disease-causing mutations on lubricin protein synthesis, secretion, and post-translational processing. *J Biol Chem* 280: 31325-31332.
- Ali L, Flowers SA, Jin C, Bennet EP, Ekwall AK, et al. (2014) The O-glycomap of lubricin, a novel mucin responsible for joint lubrication, identified by site-specific glycopeptide analysis. *Mol Cell Proteomics* 13: 3396-3409.
- Lee S, Muller M, Rezwani K, Spencer ND (2005) Porcine gastric mucin (PGM) at the water/poly(dimethylsiloxane) (PDMS) interface: Influence of pH and ionic strength on its conformation,

- adsorption and aqueous lubrication properties. *Langmuir* 21: 8344-8353.
21. Swann DA, Hendren RB, Radin EL, Sotman SL, Duda EA (1981) The lubricating activity of synovial fluid glycoproteins. *Arthritis Rheum* 24: 22-30.
 22. Jahn S, Seror J, Klein J (2016) Lubrication of articular cartilage. *Annu Rev Biomed Eng* 18: 235-258.
 23. Lorenz H, Richter W (2006) Osteoarthritis: Cellular and molecular changes in degenerating cartilage. *Prog Histochem Cytochem* 40: 135-163.
 24. Nguyen LS, Wilkinson MF, Geck J (2014) Nonsense-mediated mRNA decay: Inter-individual variability and human disease. *Neurosci Biobehav Rev* 46: 175-186.
 25. Zetoune AB, Fontaniere S, Magnin D, Anczukow O, Buisson M, et al. (2008) Comparison of nonsense-mediated mRNA decay efficiency in various murine tissues. *BMC Genet* 9: 83.
 26. Bhuvanagiri M, Schlitter AM, Hentze MW, Kulozik AE (2010) NMD: RNA biology meets human genetic medicine. *Biochem J* 430: 365-377.
 27. Thein SL, Hesketh C, Taylor P, Temperley IJ, Hutchinson RM, et al. (1990) Molecular basis for dominantly inherited inclusion body beta-thalassemia. *Proc Natl Acad Sci U S A* 87: 3924-3928.
 28. Karousis ED, Nasif S, Muhlemann O (2016) Nonsense-mediated mRNA decay: Novel mechanistic insights and biological impact. *Wiley Interdiscip Rev RNA* 7: 661-682.
 29. Bombieri C, Claustres M, De Boeck K, Derichs N, Dodge J, et al. (2011) Recommendations for the classification of diseases as CFTR-related disorders. *J Cyst Fibros* 10: S86-102.
 30. Castellani C, Assael BM (2017) Cystic fibrosis: A clinical view. *Cell Mol Life Sci* 74: 129-140.