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Ancestral analysis of a Native American Ecuadorian family with congenital insensitivity to pain with anhidrosis

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ABSTRACT

Congenital insensitivity to pain with anhidrosis (CIPA) is an extremely rare autosomal recessive disorder characterized by self-mutilating behavior, unexplained fever, inability to sweat and intellectual disability. CIPA pathogenesis is associated with genetic loss-of-function mutations of the *NTRK1* gene, which is auto phosphorylated activating intracellular signaling transduction such as cell survival, growth and differentiation. CIPA occurs with an incidence of 1 in 125 million newborns, and only some hundreds of cases have been reported worldwide. Most of cases have been reported in Asian countries. Here, we estimate the ancestral proportions of a family with consanguinity background affected with CIPA, who carries the missense pathogenic mutations rs80356677 (Asp674Tyr) in the kinase domain of *NTRK1* and rs324420 (Pro129Thr) in the *FAAH* gene. The ancestral proportion was calculated through 45 ancestry informative markers (AIMs) and the comparison was done through the Human Genome Diversity Project panel. The resulting allele frequencies in CIPA family indicate a prevalence of the Native American ancestral component with 87.9%, and minor proportion for the European (8.9%) and African (3%) components. In conclusion, the genetic variations expressing CIPA in a Native American Ecuadorian family could have been caused by the insertion of certain genetic characteristics, which have been passed down from common ancestors as consequence of migration towards South America.

1. Introduction

CIPA is an extremely rare autosomal recessive disorder characterized by axonal atrophy affecting the sensory and autonomic neurons [1]. CIPA patients have recurrent episodes of unexplained fever, self-mutilating behavior, intellectual disability, absence of reaction to noxious stimuli, anhidrosis, palmoplantar keratoderma, humoral immunodeficiency, and early onset renal disease [2]. Regarding genetics, on the one side, *NTRK1* encodes the neurotrophic tyrosine kinase-1 receptor, which is autophosphorylated in response to the *NGF*, thus, activating various pathways of intracellular signaling transduction such as cell growth, differentiation and survival [3]. These signal transduction pathways mediate innervation of the skin by sensory and sympathetic axons. Genetic alterations in *NTRK1* cause pain insensitivity by the absence of the *NGF*-dependent primary afferents [1]. On the other side, fatty-acid amide hydrolase (*FAAH*) is the major catabolic enzyme for a range of bioactive lipids. The human *FAAH* gene contains a commonly carried hypomorphic single-nucleotide polymorphism (SNP) (rs324420) that reduces the activity of FAAH enzyme and it is associated with pain sensitivity [4].

2. Materials and methods

A total of 7 individuals who live 2418 m above sea level were analyzed. Genomic DNA extraction of individuals was performed using the PureLink DNA Kit (Invitrogen). DNA was extracted from whole blood samples and presented an average concentration of 45 ng/μL, obtained using NanoDrop 2000 (Thermo Scientific).

Next Generation Sequencing (NGS): Genomic DNA of the CIPA patient was enriched by using the TruSight One NGS Panel (Illumina) and

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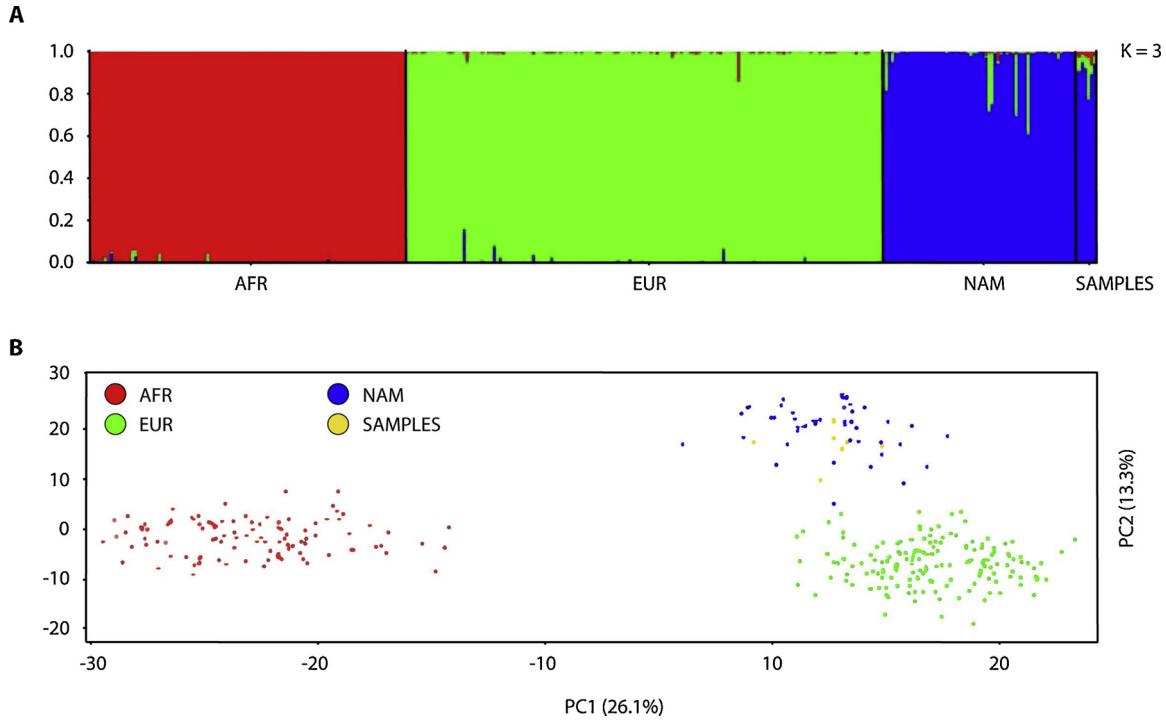

Fig. 1. Ancestry Informative Markers. A) Ancestral membership proportions. The reference samples belong to the ancestral continental populations (EUR, European; AFR, African; NAM, Native American). B) Principal component analysis of the Native American CIPA family members and reference population. Plot constructed using RStudio v1.0.44.

sequenced on the Illumina MiSeq platform. NGS analyzed 4811 genes and 18,933 variants through the BaseSpace Variant Interpreter Software (Illumina), SIFT [5], and PolyPhen-2 [6] bioinformatics tools.

Ancestry Informative Markers (AIMs): 7 samples were genotyped by single multiplex PCR using 45 autosomal AIMs described by Pereira et al. [7]. Fluorescent DNA fragments were separated and detected by capillary electrophoresis using LIZ 600 and POP7 polymer in a ABI 3130 Genetic Analyzer (Applied Biosystems), and were identified using the software GeneMapper V3.2 following allele nomenclature previously described [7].

Statistical analysis: T-student test was performed to determine the ancestral predominance between cases and controls compared to reference groups. Inference of ancestry proportions were obtained by Structure V2.3.4 software [8] using the admixture model with K = 3 (based in tri-hybrid historic mixture). Runs consisted in 100,000 burnin steps, followed by 100,000 Markov Chain Monte Carlo without taking into account group information a priori. All runs were made without any prior information on the origin of samples and only took into account the genetic background for the ancestral continental populations based on reference samples: European (EUR), African (AFR), and Native American (NAM).

3. Results

NGS analysis reveals that CIPA patient carries the pathogenic mutation rs80356677 (Asp674Tyr) in the kinase domain of *NTRK1*, and the pathogenic mutation rs324420 (Pro129Thr) in the *FAAH* gene.

Fig. 1A (Bar plot) details the ancestry analysis of the seven samples. Group 1 (red) belongs to AFR population, group 2 (green) belongs to EUR population, group 3 (blue) belongs to NAM population and group 4 belongs to CIPA family. The predominant ancestral percentage in CIPA family had 87.9% of NAM, followed by 8.9% of EUR, and 3% of AFR. Finally, Fig. 1B shows the principal component analysis (PCA) of the CIPA family and reference populations using RStudio v1.0.44.

4. Discussion

The pathogenic mutation *NTRK1* rs80356677 (Asp674Tyr) is associated with unexplained fever, inability to sweat and intellectual disability, and the pathogenic mutation *FAAH* rs324420 (Pro129Thr) is associated with pain insensitivity [4]. Regarding the ancestral analysis of the CIPA family, the highest percentage belongs to NAM, followed by EUR and AFR. However, there is an ancestral predominance of the NAM group in all samples.

5. Conclusions

This is the first time that an ancestry analysis was performed in a NAM family with consanguinity background and CIPA. Additionally, this is the first time that the *FAAH* rs324420 pathogenic mutation was identified and reported in a Native American patient with CIPA in Latin America.

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None.

Declaration of Competing Interest

None.

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References

[1] R. Altassan, H. Al Saud, T.A. Masoodi, et al., Exome sequencing identifies novel NTRK1 mutations in patients with HSAN-IV phenotype, Am. J. Med. Genet. Part A. 173 (2017) 1009–1016.
[2] Y. Indo, Nerve growth factor and the physiology of pain: lessons from congenital

insensitivity to pain with anhidrosis, Clin. Genet. 82 (2012) 341–350.
[3] A.V. Varma, L. McBride, M. Marble, et al., Congenital insensitivity to pain and anhidrosis: case report and review of findings along neuro-immune axis in the disorder, J. Neurol. Sci. 15 (2016) 201–210.
[4] A.M. Habib, A.L. Okorokov, M.N. Hill, et al., Microdeletion in a FAAH pseudogene identified in a patient with high anandamide concentrations and pain insensitivity, Br. J. Anaesth. 123 (2019) e249–e253.
[5] P.C. Ng, S. Henikoff, SIFT: predicting amino acid changes that affect protein function, Nucleic Acids Res. 31 (2003) 3812–3814.
[6] D.M. Adzhubei, S.R. Jordan, Sunyaev, Predicting functional effect of human missense mutations using PolyPhen-2, Curr. Protoc. Hum. Genet. 76 (2013) 7.20.1–7.20.41.
[7] R. Pereira, C. Phillips, N. Pinto, et al., Straightforward inference of ancestry and admixture proportions through ancestry-informative insertion deletion multiplexing, PLoS One 7 (2012) e29684.
[8] J.K. Pritchard, M. Stephens, P. Donnelly, Inference of population structure using multilocus genotype data, Genetics 155 (2000) 945–959.