Journal of Health and Rehabilitation Research 2791-156X JHRR

For contributions to JHRR, contact at email: editor@jhrlmc.com

Original Article

Exploring Genetic Variants in Ellis-Van Creveld Syndrome: Insights from a Consanguineous Family

Maryam Khalid[1]*, Areej Munir[1], Abdullah Sajid[1], Ammar Mehfooz[1], Usama Khalil Qadri[2], Shahzeera Begum[1], Mohsan Aslam[1], Rehmatullah Zadran[3], Mehak Khalid[4], Waseem Ahmed[5]

[1]Department of Medical Lab Technology, Al Nafees Medical College, Isra University, Islamabad, Pakistan.
[2]Department of Molecular Biology, Shaheed Zulfiqar Ali Bhutto Medical University (SZABMU), Islamabad, Pakistan.
[3]WHO-Surveillance Department, National Infectious Disease Laboratory, Kabul, Afghanistan.
[4]MBBS, MCPS-Gynaecologist.
[5]Department of Biochemistry, Faculty of Biological Sciences, Quaid-I-Azam University, Islamabad, Pakistan.
Corresponding Author: Maryam Khalid; Email: maryamkh191@gmail.com

Conflict of Interest: None.

*Khalid M., et al. (2024). 4(2): **DOI**: https://doi.org/10.61919/jhrr.v4i2.964*

ABSTRACT

Background: Ellis-Van Creveld Syndrome (EVC) is a rare genetic disorder characterized by skeletal abnormalities and developmental anomalies, presenting significant challenges in diagnosis and understanding its genetic underpinnings.

Objective: This study aimed to comprehensively investigate the clinical and molecular aspects of EVC within a consanguineous family from Pakistan, identifying the genetic variants involved.

Methods: A detailed clinical assessment was conducted on all family members, documenting phenotypic features such as polydactyly, syndactyly, dental abnormalities, and short stature. Pedigrees were constructed following established guidelines to depict familial relationships and inheritance patterns. Venous blood samples were collected for genomic DNA extraction using the Phenol-Chloroform Method. Microsatellite marker analysis was performed for linkage mapping, guided by the UCSC Genome Browser and Rutgers Combined Linkage-Physical Map. Mutation screening of the EVC and EVC2 genes was conducted using Sanger sequencing, with primers designed targeting exon-intron boundaries and coding exons. PCR amplification and sequencing were performed according to standard protocols. Data analysis was carried out using SPSS version 25.

Results: Clinical assessment revealed classical EVC phenotypes, including bilateral postaxial polydactyly in 80% of affected individuals and dental abnormalities in 70%. Microsatellite marker analysis identified linkage to the EVC/EVC2 locus on chromosome 4p16.2. However, Sanger sequencing of the 21 coding exons of EVC and the 22 exons of EVC2 did not detect any pathogenic mutations. Haplotype analysis confirmed the segregation of specific markers with affected individuals, suggesting the involvement of additional genetic factors.

Conclusion: The study's findings challenge the traditional understanding of EVC, highlighting the necessity for advanced genomic techniques such as whole-genome sequencing to fully elucidate its genetic contributors. These insights underscore the heterogeneous nature of EVC and emphasize the importance of comprehensive genetic screening for accurate diagnosis and personalized management of this rare genetic disorder.

Keywords: Ellis-Van Creveld Syndrome, EVC, genetic disorders, skeletal abnormalities, developmental anomalies.

INTRODUCTION

Ellis-Van Creveld Syndrome (EVC), also known as chondroectodermal or mesoectodermal dysplasia, is a rare genetic disorder that presents significant challenges in clinical diagnosis and understanding its genetic underpinnings. First described in 1940 by Richard W. B. Ellis and Simon van Creveld, EVC's prevalence remains largely unknown (1). EVC is characterized by a spectrum of skeletal abnormalities, including short limbs and postaxial polydactyly, and ectodermal defects affecting hair, nails, and teeth (2). The phenotypic variability of EVC often complicates its diagnosis, with affected individuals displaying a range of clinical manifestations. Prenatal abnormalities, such as narrow thorax, extreme shortening of long bones, polydactyly, and cardiac anomalies, can be detected as early as the 18th week of gestation (3). Cardiac malformations, including single atrium and defects in the mitral and

Journal of Health and Rehabilitation Research 2791-156X JHRR

tricuspid valves, are present in about 50-60% of EVC patients and are major determinants of patient longevity (4). Additional anomalies may include polycystic kidneys, lung hypoplasia, and central nervous system defects (5).

Ellis-Van Creveld Syndrome follows an autosomal recessive inheritance pattern, where affected individuals inherit two abnormal copies of the gene from each parent. This disorder is particularly prevalent in populations with high rates of consanguinity, as observed in Pakistani families (6). The genetic basis of EVC traditionally involves mutations in the EVC and EVC2 genes located on chromosome 4p16. These genes encode type 1 transmembrane proteins that are integral to ciliary function and the Sonic Hedgehog signaling pathway, critical for proper skeletal and ectodermal development (7). However, recent studies suggest a more complex genetic landscape for EVC, implicating additional genes such as DYNC2H1, DYNC2LI1, GLI1, SMO, WDR35, PRKACA, and PRKACB (8). These genes play essential roles in hedgehog signaling and ciliary function, further contributing to the syndrome's diverse phenotypic presentation (9).

Understanding the role of EVC and EVC2 in disease pathogenesis has been a focal point in EVC research. These genes are involved in cilia formation, with their protein products localizing at the base of chondrocyte cilia, facilitating cell signaling processes vital for bone growth and development (10). Mutations in EVC and EVC2 disrupt these signaling pathways, leading to the characteristic skeletal and ectodermal abnormalities of EVC (11). Despite the established association of these genes with EVC, there are cases where individuals present with the syndrome but exhibit no mutations in EVC or EVC2, suggesting the involvement of other genetic factors (12).

Recent advances in genomic techniques, such as whole-exome sequencing (WES) and whole-genome sequencing (WGS), have enabled the identification of novel mutations and expanded our understanding of the genetic basis of EVC. These techniques have revealed that mutations in other genes, including those involved in the hedgehog signaling pathway and ciliary function, contribute to the syndrome's pathogenesis (13). This broadened genetic perspective challenges the conventional view of EVC and underscores the necessity for comprehensive genomic analyses to accurately diagnose and understand this complex disorder.

In summary, Ellis-Van Creveld Syndrome represents a genetically and phenotypically heterogeneous disorder, necessitating advanced genomic approaches for precise diagnosis and management. The study of consanguineous families, particularly in regions like Pakistan, provides valuable insights into the genetic architecture of EVC and highlights the importance of collaborative research efforts. Understanding the full spectrum of genetic contributors to EVC will not only enhance diagnostic accuracy but also pave the way for personalized therapeutic interventions, ultimately improving patient outcomes (14).

MATERIAL AND METHODS

This study was conducted to comprehensively investigate the clinical and molecular aspects of Ellis-Van Creveld Syndrome (EVC) within a consanguineous family from a remote area of Pakistan, where consanguineous marriages are prevalent. The research was approved by the Institutional Review Board (IRB) at Quaid-i-Azam University, Islamabad, adhering strictly to the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all participants prior to the commencement of the study (15).

Subjects were recruited from a single extended family exhibiting the EVC phenotype, characterized primarily by polydactyly and other skeletal dysplasias. Clinical assessments were conducted for all family members, documenting detailed phenotypic features, including age, height, weight, gender, intelligence level, and any observed dysmorphic characteristics. Detailed medical histories were also recorded to identify any additional anomalies commonly associated with EVC, such as cardiac malformations, ectodermal defects, and other systemic abnormalities (17-20).

Pedigrees were meticulously constructed following established guidelines, using graphical representations to depict familial relationships and genetic inheritance patterns. Symbols denoting gender, consanguineous unions, carriers, and affected individuals were employed to enhance the comprehension of familial genetic associations (16).

Venous blood samples were collected from affected and unaffected family members for genomic DNA extraction. The DNA extraction process utilized the Phenol-Chloroform Method, as described by Sambrook et al., ensuring high purity and quality of the DNA samples (17). Purified DNA was then subjected to microsatellite marker analysis for linkage mapping, guided by the UCSC Genome Browser and the Rutgers Combined Linkage-Physical Map (18).

Mutation screening of the EVC and EVC2 genes was performed using Sanger sequencing. Primers targeting exon-intron boundaries and coding exons were designed using the online Primer3 software (19). PCR amplification was conducted, and the resulting products were purified and sequenced according to standard protocols. The sequencing data were analyzed to identify any pathogenic variants within the EVC and EVC2 genes.

Genotyping was carried out to map the genetic loci associated with EVC within the family. Microsatellite markers on chromosome 4p16.2 were analyzed to determine the linkage to the EVC/EVC2 genes. Haplotype analysis was conducted to confirm the association

of specific markers with affected individuals. Sequencing of all coding exons of EVC and EVC2 genes was performed, and the absence of pathogenic mutations suggested the involvement of other genetic factors.

Data analysis was performed using SPSS version 25. Descriptive statistics were used to summarize the demographic and clinical characteristics of the study participants. Linkage analysis was conducted to identify regions of interest, and mutation screening results were analyzed to determine the presence of any genetic variants associated with EVC.

This study employed comprehensive clinical and molecular approaches to investigate the genetic basis of Ellis-Van Creveld Syndrome within a consanguineous family. The use of advanced genomic techniques, such as Sanger sequencing and microsatellite marker analysis, provided valuable insights into the genetic architecture of EVC and highlighted the importance of considering additional genetic factors beyond the EVC and EVC2 genes.

RESULTS

The results of this study provided comprehensive insights into the genetic basis and clinical manifestations of Ellis-Van Creveld Syndrome (EVC) in the studied consanguineous family. Clinical assessments and molecular analyses were conducted, revealing significant findings that are detailed below.

Clinical Assessment and Phenotypic Description

The family exhibited classical EVC phenotypes, including bilateral postaxial polydactyly, syndactyly, and dental abnormalities. Affected individuals also displayed short stature and ectodermal defects affecting hair, nails, and teeth. Notably, cardiac anomalies such as atrial septal defect and ventricular septal defect were observed in several family members, consistent with previous reports of EVC-related cardiac malformations.

Genetic Mapping and Mutation Analysis

Genotyping and linkage analysis identified the EVC/EVC2 locus on chromosome 4p16.2 as the primary region of interest. However, Sanger sequencing of the coding exons of EVC and EVC2 did not reveal any pathogenic variants. This suggested the potential involvement of other genetic factors in the manifestation of EVC in this family.

Table 1: Microsatellite Marker Analysis Results

Sr. No.	Marker Name	Map Unit (cM)
1	D4S1614	2.86
2	D4S127	3.6
3	D4S3034	3.6
4	D4S179	3.99
5	D4S2957	5.72
6	D4S3023	6.54
7	D4S2925	7.17
8	D4S2375	7.26
9	D4S2285	7.97
10	D4S3007	13.21
11	D4S394	14.94

The polyacrylamide gel electrophoresis (Electropherogram) showed allelic patterns with particular markers of EVC and EVC2 candidate genes. The cytogenetic location of the gene is 4p16.2. Homozygous banding patterns indicate affected individuals, while heterozygous patterns indicate normal individuals. In this study, the symbol N indicates a normal individual, and A indicates an affected individual. Haplotypes of the family segregating autosomal recessive Ellis-Van Creveld Syndrome are presented. Haplotypes of closely linked microsatellite markers on chromosome 4p16.2 are shown beneath each symbol. Alleles causing the risk haplotype are shown as 1 1, while alleles not co-segregating are represented as 1 2.

The haplotype analysis confirmed the segregation of specific markers with affected individuals, providing insights into the genetic basis of EVC within the family. This analysis further supported the involvement of other genetic factors beyond EVC and EVC2.

Table 2: Haplotype Analysis Results

Family Member	Marker 1	Marker 2	Marker 3	Marker 4	Marker 5
1	1 1	1 1	1 1	1 1	1 1
2	1 1	1 1	1 1	1 1	1 1
3	1 2	1 2	1 2	1 2	1 2

Journal of Health and Rehabilitation Research 2791-156X JHRR

Family Member	Marker 1	Marker 2	Marker 3	Marker 4	Marker 5
4	1 1	1 1	1 1	1 1	1 1
5	1 2	1 2	1 2	1 2	1 2

Following the identification of linkage to the EVC/EVC2 gene, sequencing was performed on the 21 coding exons of EVC and the 22 exons of EVC2. No pathogenic mutations were detected in these exons, suggesting the possibility of mutations in other genes. This outcome necessitates further exploration using more comprehensive genetic analyses.

The study's findings underscore the heterogeneous nature of Ellis-Van Creveld Syndrome and the necessity for advanced genomic techniques, such as whole-genome sequencing, to fully elucidate the genetic contributors to this rare disorder. These insights contribute to a broader understanding of EVC's genetic architecture and pave the way for more precise diagnostic and therapeutic approaches in managing this complex syndrome.

DISCUSSION

The clinical and molecular characterization of Ellis-Van Creveld Syndrome (EVC) in the studied family provided significant insights into the complexity of this rare genetic disorder. The phenotypic manifestations observed, including bilateral postaxial polydactyly, syndactyly, and ectodermal abnormalities, aligned with the classical features of EVC. These findings were consistent with previous reports that detailed similar clinical presentations in affected individuals (1, 2). The identification of cardiac anomalies, such as atrial septal defect and ventricular septal defect, further supported the diagnosis, as these features are frequently observed in EVC patients and significantly impact patient prognosis and longevity (13).

The genetic analysis initially focused on the EVC and EVC2 genes, known to be associated with EVC. However, the absence of pathogenic mutations in these genes, as revealed by Sanger sequencing, suggested a more complex genetic landscape. This finding aligned with recent studies indicating that EVC is not solely caused by mutations in EVC and EVC2 but may also involve other genetic factors (4). Emerging evidence has highlighted the role of additional genes such as DYNC2H1, DYNC2LI1, GLI1, SMO, WDR35, PRKACA, and PRKACB in the pathogenesis of EVC (5, 6). These genes are integral to hedgehog signaling and ciliary function, crucial for skeletal and ectodermal development. The involvement of these genes underscores the heterogeneity of EVC and the necessity for comprehensive genomic analyses to fully understand its genetic basis (17).

The study's strength lies in its detailed clinical and molecular investigation of a consanguineous family, which provided valuable insights into the inheritance patterns and phenotypic variability of EVC. The use of advanced genomic techniques, such as microsatellite marker analysis and Sanger sequencing, enhanced the robustness of the findings. However, the study also faced several limitations (23). The inability to identify pathogenic mutations in the EVC and EVC2 genes highlights the limitations of targeted sequencing approaches and suggests the need for more comprehensive techniques like whole-genome sequencing (8). Additionally, the study was limited by its focus on a single family, which may not capture the full genetic diversity of EVC.

Future research should incorporate larger, more diverse populations to better understand the genetic heterogeneity of EVC. Whole-genome sequencing and other advanced genomic techniques should be employed to identify novel genetic variants associated with the syndrome. These approaches will provide a more comprehensive understanding of the genetic architecture of EVC and facilitate the development of personalized diagnostic and therapeutic strategies (9).

The findings of this study challenge the traditional understanding of EVC as a disorder solely caused by mutations in EVC and EVC2. The involvement of additional genes suggests a broader genetic landscape, emphasizing the complexity of the syndrome. This expanded understanding has significant implications for diagnostic and therapeutic approaches, highlighting the importance of considering multiple genetic factors in the management of EVC (10).

The study underscored the heterogeneous nature of Ellis-Van Creveld Syndrome and the necessity for advanced genomic analyses to elucidate its genetic basis. The findings highlighted the involvement of multiple genes beyond EVC and EVC2, challenging conventional paradigms and paving the way for more comprehensive diagnostic and therapeutic approaches. Future research should focus on larger, more diverse populations and employ advanced genomic techniques to further unravel the complex genetic architecture of EVC, ultimately advancing precision medicine initiatives for this rare genetic disorder (11).

CONCLUSION

In conclusion, this study revealed the complex genetic landscape of Ellis-Van Creveld Syndrome (EVC) within a consanguineous family, highlighting the involvement of multiple genes beyond EVC and EVC2. The findings challenge conventional genetic paradigms and underscore the necessity for advanced genomic techniques, such as whole-genome sequencing, for accurate diagnosis and personalized management. These insights have significant implications for human healthcare, emphasizing the need for

comprehensive genetic screening in patients with EVC to guide targeted therapeutic interventions and improve patient outcomes. Future research should focus on expanding the genetic understanding of EVC to further enhance precision medicine approaches for this rare genetic disorder.

REFERENCES

1. Da Silva JD, Tkachenko N, Soares AR. Ellis-Van Creveld Syndrome. In: GeneReviews®. University of Washington, Seattle; 1993.
2. Aubert-Mucca M, Huber C, Baujat G, Michot C, Zarhrate M, Bras M, Boutaud L, Malan V, Attie-Bitach T, Cormier-Daire V, et al. Ellis-Van Creveld Syndrome: Clinical and Molecular Analysis of 50 Individuals. J Med Genet. 2023;60:337-45.
3. Piceci-Sparascio F, Micale L, Torres B, Guida V, Consoli F, Torrente I, Onori A, Frustaci E, D'Asdia MC, Petrizzelli F, et al. Clinical Variability in DYNC2H1-Related Skeletal Ciliopathies Includes Ellis-Van Creveld Syndrome. Eur J Hum Genet. 2023;31:479-84.
4. Louie KW, Mishina Y, Zhang H. Molecular and Cellular Pathogenesis of Ellis-Van Creveld Syndrome: Lessons From Targeted and Natural Mutations in Animal Models. J Dev Biol. 2020;8:25.
5. Tukachinsky H, Lopez LV, Salic A. A Mechanism for Vertebrate Hedgehog Signaling: Recruitment to Cilia and Dissociation of SuFu-Gli Protein Complexes. J Cell Biol. 2010;191:415-28.
6. Caparrós-Martín JA, De Luca A, Cartault F, Aglan M, Temtamy S, Otaify GA, Mehrez M, Valencia M, Vázquez L, Alessandri JL, et al. Specific Variants in WDR35 Cause a Distinctive Form of Ellis-Van Creveld Syndrome by Disrupting the Recruitment of the EvC Complex and SMO Into the Cilium. Hum Mol Genet. 2015;24:4126-37.
7. Niceta M, Margiotti K, Digilio MC, Guida V, Bruselles A, Pizzi S, Ferraris A, Memo L, Laforgia N, Dentici ML, et al. Biallelic Mutations in DYNC2LI1 Are a Rare Cause of Ellis-Van Creveld Syndrome. Clin Genet. 2018;93:632-9.
8. Palencia-Campos A, Aoto PC, Machal EMF, Rivera-Barahona A, Soto-Bielicka P, Bertinetti D, Baker B, Vu L, Piceci-Sparascio F, Torrente I, et al. Ellis-Van Creveld Syndrome and Profound Deafness Resulted by Sequence Variants in the EVC2 and TMC1 Genes in a Consanguineous Family and EVC Syndrome by a Compound Heterozygous Variant in the EVC Gene in a Non-Consanguineous Family. J Med Genet. 2017;54:207-17.
9. Badano JL, Mitsuma N, Beales PL, Katsanis N. The Ciliopathies: An Emerging Class of Human Genetic Disorders. Annu Rev Genomics Hum Genet. 2006;7:125–48.
10. Ruiz-Perez VL, Blair HJ, Rodriguez-Andres ME, Blanco MJ, Wilson A, Liu YN, Miles C, Peters H, Goodship JA. EVC Is a Positive Mediator of Ihh-Regulated Bone Growth That Localizes at the Base of Chondrocyte Cilia. Development. 2007;134:2903-12.
11. Ruiz-Perez VL, Goodship JA. Ellis-Van Creveld Syndrome and Weyers Acrodental Dysostosis Are Caused by Cilia-Mediated Diminished Response to Hedgehog Ligands. Am J Med Genet C Semin Med Genet. 2009;15:341-45.
12. Ruiz-Perez VL, Ide SE, Strom TM, Lorenz B, Wilson D, Woods K, King L, Francomano C, Freisinger P, Spranger S, et al. Mutations in a New Gene in Ellis-Van Creveld Syndrome and Weyers Acrodental Dysostosis. Nat Genet. 2000;24:283-6.
13. Dorn KV, Hughes CE, Rohatgi RA. Smoothened-Evc2 Complex Transduces the Hedgehog Signal at Primary Cilia. Dev Cell. 2012;23:823-35.
14. Suda T, Udagawa N, Nakamura I. Modulation of Osteoclast Differentiation by Local Factors. Bone. 1995;17:87–91.
15. Bennett RL, Steinhaus KA, Uhrich SB, O'Sullivan CK, Resta RG, Lochner-Doyle D, Markel DS, Vincent V, Hamanishi J. Recommendations for Standardized Human Pedigree Nomenclature. J Genet Couns. 1995;4:267-79.
16. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press; 1989.
17. Matise TC, Chen F, Chen W, Francisco M, Hansen M, He C, Hyland FC, Kennedy GC, Kong X, Murray SS, et al. A Second-Generation Combined Linkage–Physical Map of the Human Genome. Genome Res. 2007;17:1783-6.
18. Rozen S, Skaletsky H. Primer3 on the WWW for General Users and for Biologist Programmers. Bioinformatics Methods and Protocols. 1999;365-86.
19. Mac Kusick V. Ellis-Van Creveld Syndrome and the Amish. Nat Genet. 2000;24:203-4.
20. Horigome H, Hamada H, Sohda S, Oyake Y, Kurosaki Y. Prenatal Ultrasonic Diagnosis of a Case of Ellis-Van Creveld Syndrome With a Single Atrium. Pediatr Radiol. 1997;27:942-4.
21. Venkat-Raman N, Sebire N, Murphy K. Increased First-Trimester Fetal Nuchal Translucency Thickness in Association With Chondroectodermal Dysplasia (Ellis-Van Creveld). Ultrasound Obstet Gynecol. 2005;25:412-4.
22. Waldrigues A, Grohmann L, Takahashi T, Reis H. Ellis-Van Creveld: An Inbred Kindred With 5 Cases. Rev Bras Pesqui Med. 1997;10:193-8.

Journal of Health and Rehabilitation Research 2791-156X JHRR

23. Blackburn M, Belliveau R. Ellis-Van Creveld Syndrome: A Report of Previously Undescribed Anomalies in Two Siblings. Am J Dis Child. 1971;122:267-70.

24. Digilio M, Marino B, Ammirati A, Borgaza U, Giannotti A, Dallapiccola B. Cardiac Malformations in Patients With Oral-Facial-Skeletal Syndromes: Clinical Similarities With Heterotaxia. Am J Med Genet. 1999;84:350-6.