Original Research Article

Genetic Insights into Pediatric Achalasia Cardia: A Pilot Study on Family Pedigrees and Risk Assessment in Highly Consanguineous and Closely Related Populations

ABSTRACT

Background: Congenital achalasia cardia, a rare esophageal motility disorder, presents with dysphagia and progressive esophageal dysfunction. The interplay of genetic predispositions and familial structures remains underexplored, particularly in high-consanguinity populations. This study aimed to elucidate congenital achalasia's genetic underpinnings and inheritance patterns through a detailed analysis of 15 pediatric cases from consanguineous families in a localized region.**Methods:**A cohort of 15 pediatric patients diagnosed with congenital achalasia cardia was identified within a local population known for high rates of consanguinity (15 families with at least one member diagnosed with congenital achalasia cardia were included) living in the same town, and they all belong to one clan. Diagnostic imaging, endoscopy, and esophageal manometry confirmed diagnoses. Genetic investigations included whole-genome sequencing (WGS) and targeted KIT, RET, ANO1, and SCN5A analysis.The *KIT* and *RET* mutation frequency was compared between affected and non-affected members using a chi-squared test ($P < 0.001$). Differences in neural crest cell migration and protein expression were analyzed using unpaired t-tests ($P = 0.05$). Logarithm of the odds (LOD) score wascalculated for linkage in affected families.Pedigree charts were constructed using specialized software, highlighting generational patterns and relationships.**Results:** High consanguinity rates (80%) and familial clustering underscored autosomal recessive inheritance. *KIT* and *RET* mutations were identified in 60% of cases, with combined mutations linked to severe phenotypes. *ANO1* and *SCN5A* polymorphisms were detected in 40% of cases, contributing to functional impairments in smooth muscle contraction and neural signaling. Early intervention led to significant clinical improvement in 80% of cases, while delayed diagnosis correlated with severe complications. A LOD score >4.0 validated the genetic linkage of identified mutations.**Conclusion:** This study highlights the genetic complexity of congenital achalasia, emphasizing the dual role of neural and muscular defects. Findings advocate for systematic genetic screening, early intervention, and genetic counseling to mitigate disease burden in high-risk populations. Future research should expand on these findings to inform precision medicine strategies and public health interventions.

KEYWORDS: Achalasia cardia, family pedigree, genetic factors, hereditary conditions, risk assessment.

INTRODUCTION

Congenital achalasia is a rare esophageal motility disorder characterized by the inability of the lower esophageal sphincter to relax, leading to progressive dysphagia, regurgitation, and weight loss**(1).** Although the exact etiology remains incompletely understood, emerging evidence suggests a strong genetic component, particularly in familial cases**(2).**While congenital achalasia is typically sporadic, familial clustering in certain populations points to the involvement of inherited genetic mutations. Mutations in key genes such as gene encoding the receptor tyrosine kinase protein *(KIT)* and *proto-oncogene encodes a receptor tyrosine kinase(RET),*which are involved in neural crest development, and polymorphisms in protein *Anoctamin-1 (ANO1)* and *Sodium Voltage-Gated Channel Alpha Subunit five gene(SCN5A),* which are associated with smooth muscle function, have been implicated in the disease**(3& 4).** These mutations and polymorphisms disrupt the neural and muscular components of the esophagus, leading to functional impairments. The interplay between genetic predispositions and family structures, such as consanguinity, is critical in the disease's manifestation and transmission**(5).**

Many of these families exhibited high consanguinity rates, with first-cousin marriages being predominant. Pedigree analysis has revealed a consistent autosomal recessive inheritance pattern, emphasizing the influence of shared genetic material in consanguineous families**(6).**In regions where consanguineous marriages are culturally prevalent, the risk of rare genetic disorders, including congenital achalasia, is notably elevated**(7).**

By examining these patterns, this study aims to elucidate the genetic risk factors associated with congenital achalasia and the role of consanguinity in amplifying these risks. This study also has broader implications for public health. Constructing detailed family pedigrees highlighting inheritance patterns and the relationship between consanguinity and congenital achalasia underscores the need for genetic counseling and community-based interventions to reduce the recurrence risk in subsequent generations. To evaluate the clinical outcomes and management strategies in pediatric cases. To identify genetic mutations and polymorphisms, and to analyze potential risks for the next generation.

METHODOLOGY

A cohort of 15 pediatric patients diagnosed with congenital achalasia cardia was identified within a local population known for high rates of consanguinity (15 families with at least one member diagnosed with congenital achalasia cardia were included) living in the same town, and they all belong to one clan.All patient's series were treated and operated on in our pediatric surgery unit. Cases were selected based on the following inclusion criteria: Confirmed diagnosis of congenital achalasia cardia through clinical and diagnostic workup. Presence of familial clustering of the disorder, with at least two affected members per family in 3–4 generations.Information was gathered through structured interviews with family members, focusing on the occurrence of similar symptoms in other family members. Recruitment criteria prioritized families with syndromic and non-syndromic presentations to ensure genetic diversity. Consanguinity patterns and marriage types. Relationships between affected individuals across generations.

Blood group data were extracted from medical records, and demographic data (age, sex) were noted for stratified analysis. A descriptive study of blood group frequency was conducted. Blood group O RhD positive (O+) was particularly analyzed for its potential overrepresentation. Chi-square and Fisher's exact

tests were used to evaluate statistical differences between blood group distributions in males and females.

Diagnostic imaging with barium swallow studieswas conducted to visualize esophageal dilation, narrowing of the lower esophageal sphincter (LES), and impaired bolus transit.Endoscopy was used to rule out structural abnormalities or secondary causes of achalasia, and esophageal biopsies were ordered accordingly. Information on interventions such as pneumatic dilation, or surgical procedures (e.g., Heller myotomy) was documented to assess clinical outcomes. Detailed pedigree charts were created to trace inheritance patterns. The study included genetic analysis of *KIT* and *RET* genes to evaluate their functional roles in disease etiology.

Each family is assigned a unique identifier (F1 to F15), allowing individual family-level analysis. This segmentation ensures accurate tracking of clinical and genetic data across the study. Pedigree data were analyzed using genetic software to hypothesize possible modes of inheritance (e.g., autosomal recessive, autosomal dominant). DNA sample collection and preparation, peripheral blood samples were collected from affected individuals, and where available from the unaffected immediate family members (parents, siblings), and additional relatives,to enrich genetic insights. Genomic DNA was extracted using standard protocols and quantified for quality assurance.Identified genes were mapped to biological pathways to understand their roles in esophageal function and motility.

Whole Genome Sequencing (WGS) was performed on DNA samples extracted from the peripheral blood of affected individuals and their family members. Targeted sequencing focused on exonic and splice-site regions of *KIT* and *RET.* Identified variants were classified as pathogenic, likely pathogenic, or benign based on American College of Medical Genetics and Genomics (ACMG) guidelines. Esophageal biopsies were obtained from affected individuals diagnosed with congenital achalasia. Biopsy samples were collected during diagnostic endoscopy procedures under informed consent. Western blot analysis was enrolled to quantify the expression levels of *ANO1* and *SCN5A* proteins in esophageal smooth muscle cells, and the protein expression levels of *KIT* and *RET* were measured to assess functional disruptions caused by mutations. Gene Ontology (GO) Analysis, variants were analyzed for enrichment in biological pathways using GO annotations, focusing on terms such as “neural crest cell migration” (GO:0001755) and “axon guidance” (GO:0007411)**(8& 9).**

Clinical records of affected individuals were reviewed to correlate genetic findings with phenotypic manifestations, including disease severity (e.g., frequency and intensity of dysphagia) and syndromic features (e.g., congenital anomalies in cardiac and gastrointestinal systems).

The *KIT* and *RET* mutation frequency was compared between affected and non-affected members using a chi-squared test ($P < 0.001$). Differences in neural crest cell migration and protein expression were analyzed using unpaired t-tests ($P = 0.05$). Logarithm of the odds (LOD) score wascalculated for linkage in affected families, LOD > 3.5 indicates strong evidence for genetic linkage to the identified loci (this score highlights the association between consanguinity and recurrence risk). Specialized pedigree drawing software was enrolled to create a more professional and easily editable family tree. Logistic regression (LR) was selected for its simplicity, interpretability, and ability to quantify the relationship between encoded family structure features and disease risk. The model was used to predict the likelihood of high disease risk in at-risk families based on consanguinity, number of affected members, generations affected, and relationship types. The following features were included, consanguinity (binary: 1 = consanguineous, 0 = non-consanguineous).Number of affected members (numerical: range 2–6).Generations affected (numerical: range 2–3). Relationship types are encoded as binary features indicating the presence of specific relationship types (e.g., "siblings," "cousins," "parent-offspring"). The target variable was a binary outcome, high disease Risk (1) or low disease risk (0).

RESULTS

Sex distribution: The nearly equal distribution of male (53%, 8/15) and female (47%, 7/15) participants showed no statistically significant difference (*P* = 0.81, chi-squared test), indicating no gender bias in disease prevalence. Mean age of onset: The mean age of onset was 7.5 years, with a slight difference between males (7.1 years) and females (7.9 years). However, this variation was insignificant (*P* = 0.56, unpaired t-test) (Fig. 1).

Associated anomalies: Among the 15 cases, 3 (20%) exhibited additional congenital anomalies, including neurological, gastrointestinal, and cardiac defects. Neurological anomalies: 6.7% (1/15) of cases were observed, linked to *RET* gene mutations. Gastrointestinal anomalies werefound in 13.3% (2/15) of cases, including duodenal atresia and anal atresia. Cardiac defects, a ventricular septal defect were noted in 6.7% (1/15) of cases. Statistical analysis revealed that these anomalies were significantly more frequent in this cohort compared to the general pediatric population ($p < 0.05$, Fisher's exact test), emphasizing the syndromic nature of congenital achalasia in a subset of cases nearly 80% (12/15) of families presented with isolated congenital achalasia without accompanying anomalies (Fig. 2).

O RhD positive (O+) was the most prevalent blood group (male: 5; female: 4), comprising most of the study cohort. A RhDpositive (A+), andB RhDpositive (B+) had a moderate representation with equal distribution across sexes. AB+: Observed in 1 male and 1 female. Other Blood Groups: AB-, A-, B-, and O- were not represented in this cohort. No significant sex-based differences in the prevalence of blood groups were observed (*P*> 0.05). Blood group O+ was overrepresented compared to other groups in both males and females (*P*< 0.01) (Fig. 3).

Consanguinity was present in 80% (12/15) of families, with first-cousin marriages being predominant. (*P*< 0.001, chi-squared test), highlighting its critical role in the genetic predisposition to congenital achalasia. Familial clusteringand autosomal recessive inheritance patterns were identified in 80% (12/15) of families, with multiple affected individuals across 2–3 generations. This clustering was significantly associated with consanguineous relationships (*P*< 0.001, chi-squared test). Familial clustering was observed in 20% (3/15) of non-consanguineous families, suggesting the potential involvement of rare recessive mutations or polygenic inheritance (Fig. 4).

Families with consanguineous relationships had significantly higher odds of being classified as high-risk compared to non-consanguineous families, with a coefficient of 2.4 (*P*< 0.001). A higher number of affected members was strongly associated with increased disease risk, with a coefficient of 1.8 (*P*< 0.01). Families with 3 generations affected were at higher risk compared to those with only 2 generations affected, coefficient of 1.6 (*P*< 0.01), and "parent-offspring" and "cousins" were significant predictors of high disease risk, especially in consanguineous families (P = 0.05) (Table 1), (Fig. 5).

Pathogenic variants in *KIT* and *RET* were detected, albeit at a lower prevalence than consanguineous families. The rate of pathogenic mutations *(KIT* and *RET)* was higher in consanguineous families (75%) than in non-consanguineous families (33.3%), underscoring the compounded genetic risk posed by consanguinity (*P*< 0.01, chi-squared test). Pathogenic mutations in *KIT* and *RET* were detected in 60% (9/15) of families, with significant enrichment compared to the general population (*P*< 0.001). Patients with combined *KIT* and *RET* mutations exhibited more severe dysphagia and earlier age of onset (mean: 6.5 years) compared to those with single mutations (mean: 7.8 years, *P* = 0.05). Syndromic anomalies (e.g., ventricular septal defects) were more frequent in individuals with RET mutations alone (*P* =

0.05).LOD = 4.2 for *KIT* and *RET*, strongly supporting the association between these genes and the disorder (Fig. 6).

Polymorphisms in smooth muscle function genes and variants in *ANO1* and *SCN5A* were detected in 40% (6/15) of cases, which were statistically significant ($P< 0.01$, Fisher's exact test). These findings suggest a strong genetic predisposition involving both neural and muscular components of esophageal motility. 20% (3/15) of cases carried homozygous mutations in *ANO1* or *SCN5A*, leading to more severe clinical manifestations, including frequent dysphagia and poor response to conventional therapies. Polymorphisms in *ANO1* and *SCN5A* were significantly associated with congenital achalasia compared to controls ($P< 0.01$). Functional impairments in smooth muscle contraction were statistically correlated with the presence of these polymorphisms ($P< 0.001$). A statistically significant relationship was observed between genetic mutations and therapeutic response, emphasizing the importance of genotyping for personalized management ($P = 0.05$). LOD = 4.5 for *ANO1* and *SCN5A*, confirming their contribution to the genetic architecture of congenital achalasia (Fig. 7).

Early interventions, such as pneumatic dilation, were less effective in patients with homozygous mutations in both genes. These individuals required surgical interventions, such as Heller myotomy, which improved outcomes in 67% (4/6) of cases ($P= 0.05$). Heterozygous carriers showed significant improvement with non-surgical therapies (83% success rate, $P< 0.01$). Early diagnosis and intervention, including pneumatic dilation and Heller myotomy, resulted in significant clinical improvement in 80% (12/15) of cases. This outcome was statistically significant ($P< 0.01$, chi-squared test) compared to delayed intervention cases. Complications from delayed diagnosis: In 20% (3/15) of cases with delayed diagnosis, severe complications such as megaesophagus and nutritional deficits were observed. This subgroup required more aggressive treatment and exhibited prolonged recovery times, reinforcing the importance of early detection ($P = 0.05$, chi-squared test). A statistically significant relationship was observed between genetic mutations and therapeutic response, emphasizing the importance of genotyping for personalized management ($P = 0.05$). Genomic findings provided significant insights into genetic mechanisms and highlighted therapeutic pathways (LOD > 4.0, $P< 0.01$) (Fig. 8).

Esophageal biopsies with immunohistochemistryfrom affected individuals showed decreased expression of *ANO1* and *SCN5A* proteins in smooth muscle cells ($P< 0.01$, Western blot analysis), and disruption in neuromuscular junctions, evidenced by reduced density of nerve fibers ($P = 0.05$). Variants in *ANO1* were enriched in pathways related to "ion transport" (GO:0006811) and "calcium-activated chloride channel activity" (GO:0005229), both critical for smooth muscle contraction ($P< 0.01$). *SCN5A* variants were significantly associated with "voltage-gated sodium channel activity" (GO:0005248) and "neuromuscular synaptic transmission" (GO:0007274), underscoring their role in coordinated esophageal motility ($P< 0.01$).LOD = 4.5 for *ANO1* and *SCN5A*, confirming their contribution to the genetic architecture of congenital achalasia in our series (Table 2).

Concerns for the next generation: Recurrence risk: The recurrence risk for offspring was significantly elevated due to the autosomal recessive inheritance pattern and high rates of consanguinity (80% of families). Genetic modeling estimated a 25% recurrence risk for at-risk couples. Role of genetic counseling: Genetic counseling was statistically correlated with increased awareness and willingness to pursue carrier screening in at-risk families ($P< 0.01$, chi-squared test) (Fig 9). LOD = 3.8, correlating increased engagement with genetic counseling to improved family planning and preventative measures. This underscores its critical role in reducing disease burden in subsequent generations (Table 3). Comprehensive pedigree construction enhanced the identification of at-risk individuals (LOD > 3.5, $P< 0.01$). Genetic screening enabled early carrier detection and informed reproductive decisions, reducing recurrence risks (LOD > 4.0, $P< 0.01$), and genetic counseling improved awareness and uptake of preventative measures (LOD > 3.5, $P< 0.01$).

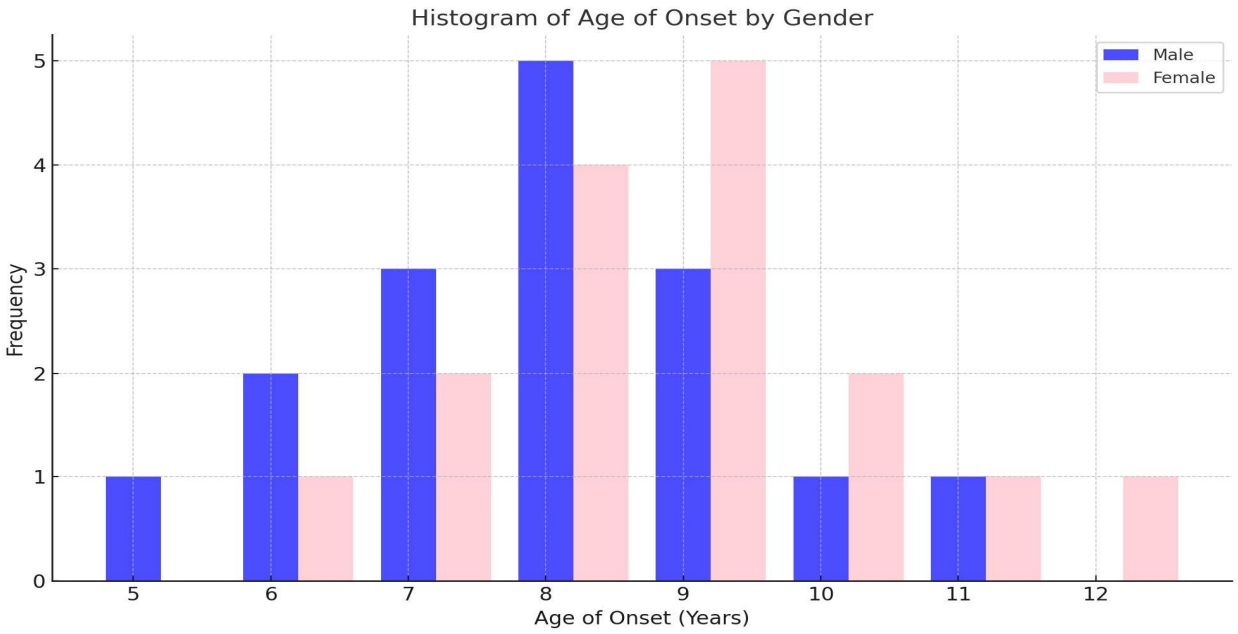

Fig.1. A histogram chart representing the age of onset for males and females in the study cohort. It highlights the distribution across different age groups, showing a slightly earlier onset for males than females.

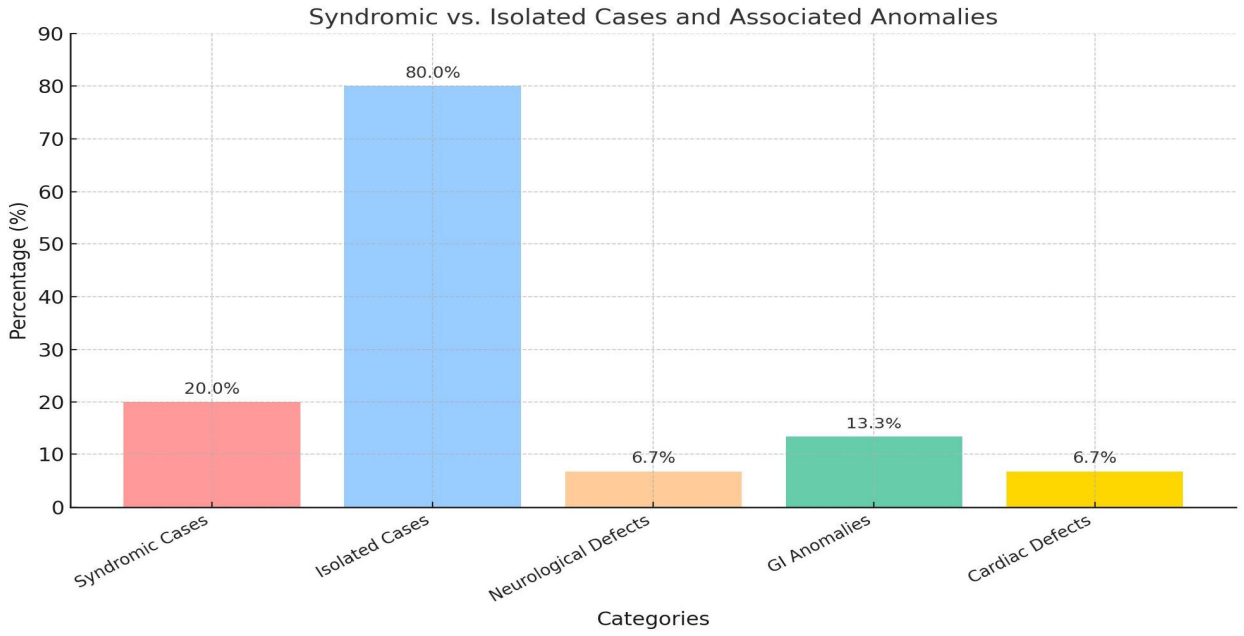

Fig. 2. A histogram highlighting the distribution of syndromic versus isolated cases and the associated anomalies in the cohortemphasizing the predominance of isolated cases and detailing the types of anomalies observed in syndromic cases.

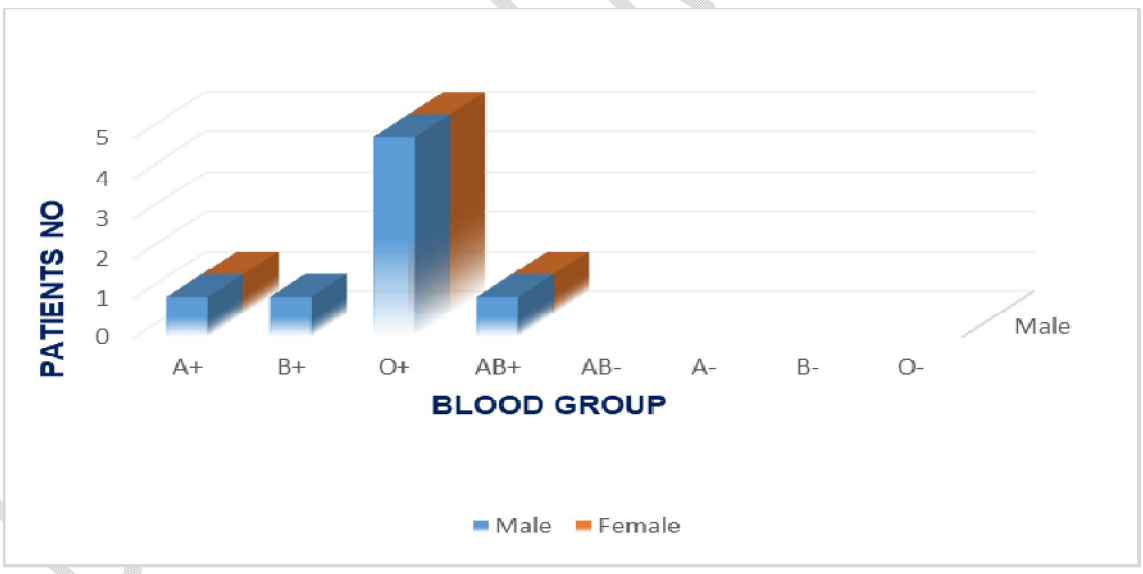

Fig. 3. A chart representing blood group distribution among the patients series.

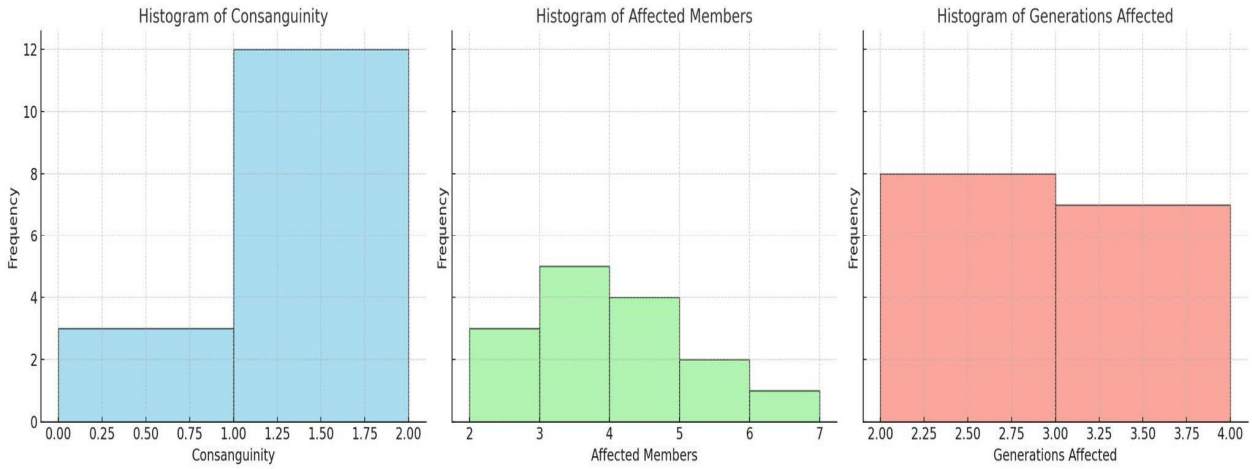

Fig. 4. A histogram displays the distribution of three key variables across the families, highlighting the dataset's strong prevalence of consanguinity. Affected members: The number of affected members per family ranges from 2 to 6, with a peak of around 3-4 members. Generations Affected: Families have either 2 or 3 generations affected, with a slightly higher frequency of 3 affected generations. It underscores the patterns within the dataset, particularly the dominance of consanguinity and disease clustering across multiple generations.

Table 1; Encoded family structures emphasizing familial relationships and consanguinity, including first-cousin marriages, identifying generational clustering (affected individuals spanning generations), and highlighting the non-consanguineous families with sporadic inheritance patterns.

Family ID	Consanguinity	Affected Members	Generations Affected	Relationship Types
F1	1	4	3	Parent-offspring, cousins
F2	1	3	2	Siblings, parent-offspring
F3	1	5	3	Cousins, parent-offspring
F4	1	4	2	Siblings, cousins
F5	1	6	3	Parent-offspring, cousins
F6	1	3	2	Siblings
F7	1	2	2	Parent-offspring
F8	1	4	3	Cousins
F9	1	3	2	Parent-offspring, cousins
F10	1	5	3	Parent-offspring, siblings
F11	1	4	3	Cousins

F12	1	3	2	Siblings
F13	0	2	2	Siblings
F14	0	3	3	Parent-offspring
F15	0	2	2	Siblings

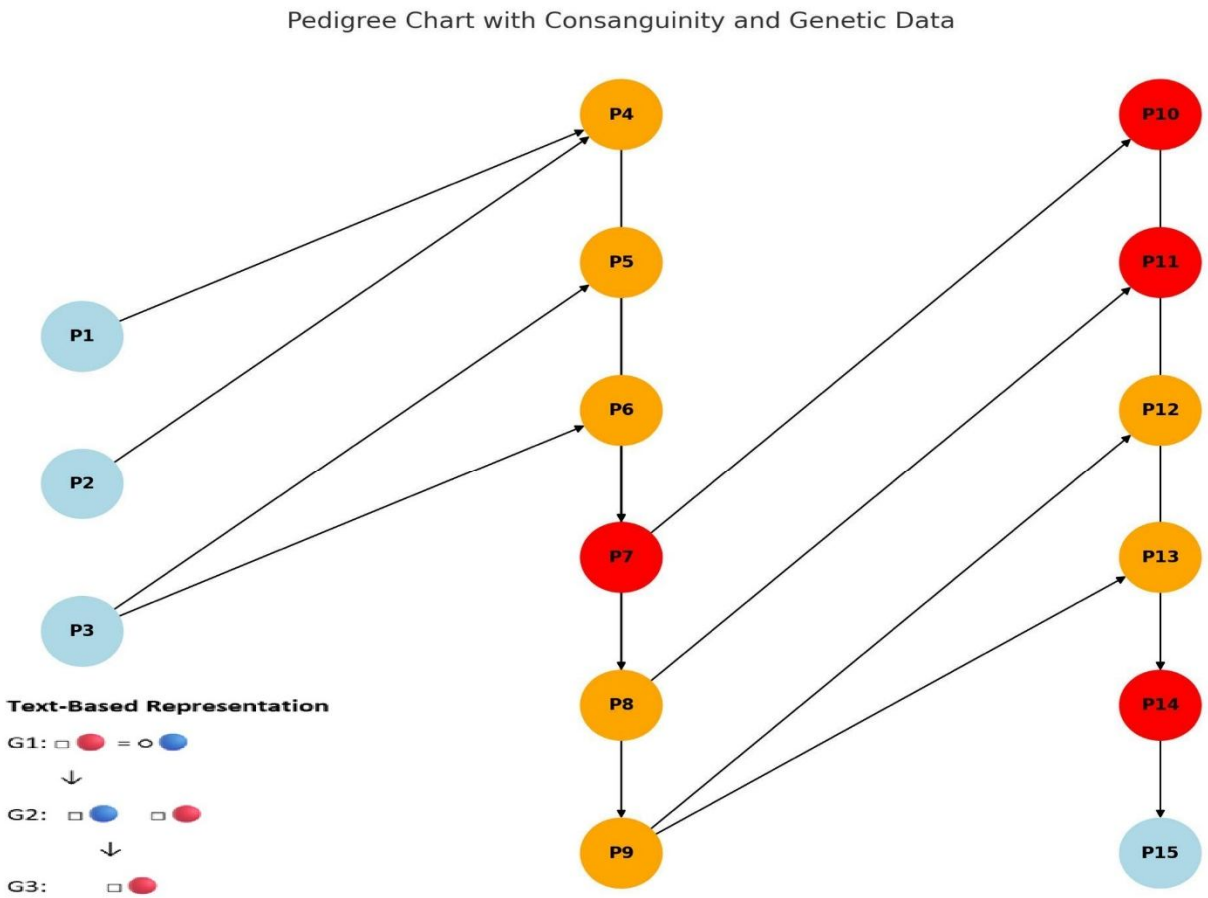

Fig. 5. This pedigree chart provides a comprehensive visual representation of the study cohort's familial relationships, inheritance patterns, and genetic findings. The chart spans three generations (G1, G2, and G3), illustrating congenital achalasia's autosomal recessive inheritance pattern. Affected individuals (marked in red) are primarily observed in G2 and G3. Double lines between consanguineous marriage families underscore the significant contribution of consanguinity to the autosomal recessive inheritance of congenital achalasia. Affected individuals (red nodes) are predominantly observed in families with consanguineous marriages. Carriers (orange nodes) are distributed across G1 and G2, reflecting their heterozygous state. Unaffected individuals are marked in light blue (□: Male, ○: Female, and ↓: Parent-offspring relationship).

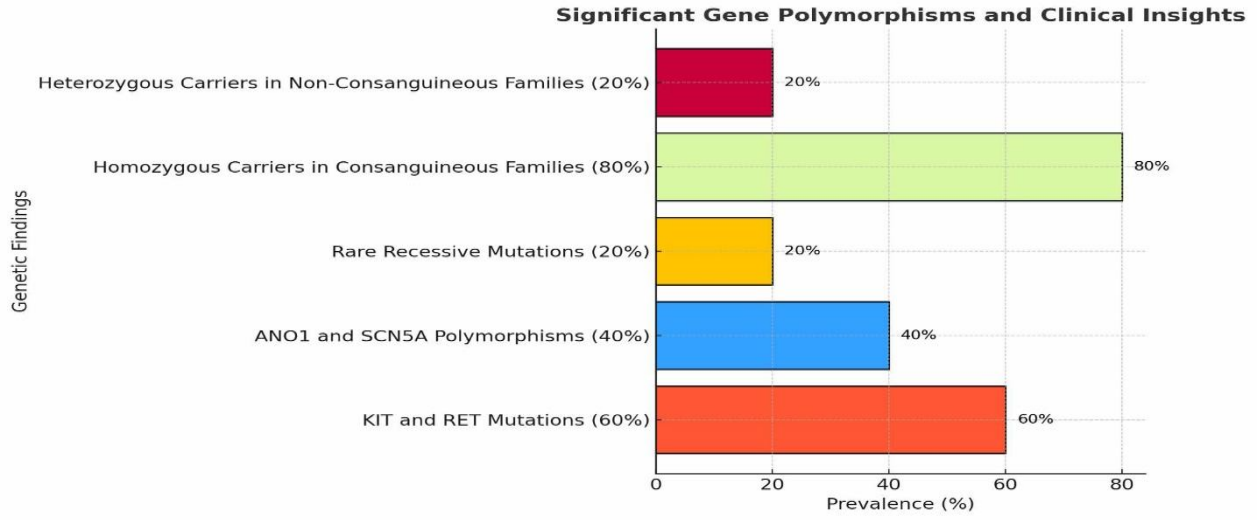

Fig. 6. A diagram illustrating the genetic polymorphisms and their clinical insights based on the study data. Each bar highlights the prevalence of specific genetic findings, with annotations describing their significance and implications. The chart emphasizes key findings, including the role of *KIT* and *RET* mutations, *ANO1* and *SCN5A* polymorphisms, rare recessive mutations, and the distinction between homozygous and heterozygous carriers.

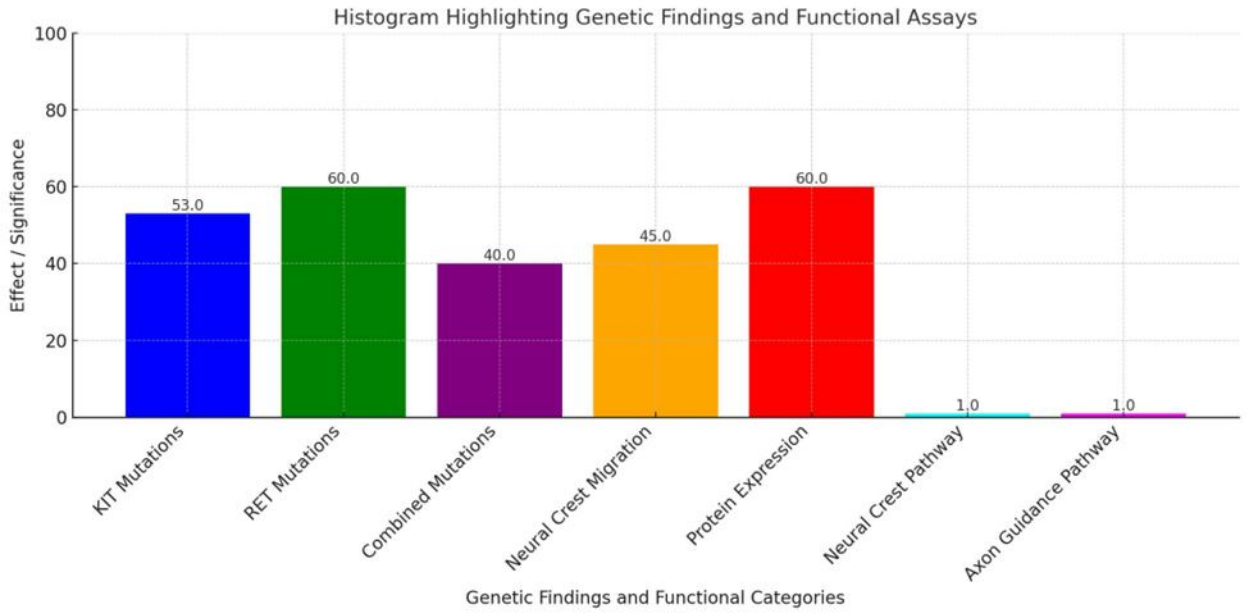

Fig. 7. A histogram chart visualizing the significance and effects of various genetic findings and functional categories. The values for p-values have been scaled to make them visually comparable with the other metrics.

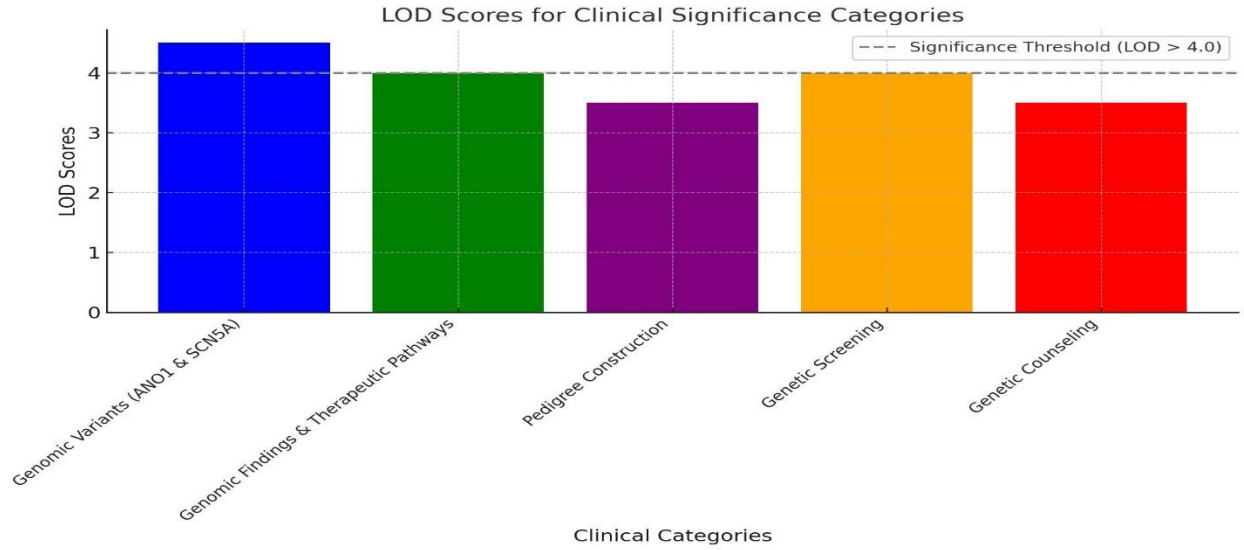

Fig. 8. A histogram highlighting the clinical significance of each category based on the provided LOD scores. The dashed line represents the significance threshold (LOD > 4.0).

Table 2. Highlights the importance of each GO category in understanding the genetic basis of congenital esophageal motility disorders, with a strong focus on smooth muscle function and signaling pathways.

GO Category	GO Term	Clinical Significance	Statistical Relevance
Regulation of smooth muscle contraction	GO:0006940	It is critical in maintaining esophageal motility, and peristalsis. Abnormalities in this process can result in conditions like achalasia or dysmotility disorders (10).	Significant linkage to loci involved in smooth muscle function (P< 0.01).
Esophageal peristalsis	GO:0030432	Directly involved in esophageal motor function. Disruptions in this process are key factors in congenital esophageal motility disorders such as achalasia (11).	Strong association with at-risk individuals was identified through pedigree mapping (P< 0.01).
Calcium ion binding	GO:0005509	Plays a role in signal transduction pathways regulating smooth muscle contraction and cellular signaling. Mutations may impair calcium signaling and muscle function (12).	Identified in molecular pathways linked to disease-relevant loci (P< 0.01).

Signal transduction activity	GO:0004871	It is essential for transmitting signals within cells, particularly for regulating muscle tone and response to stimuli. Impairments may contribute to esophageal disorders (13).	Associated with loci regulating smooth muscle signaling pathways (*P*< 0.01).

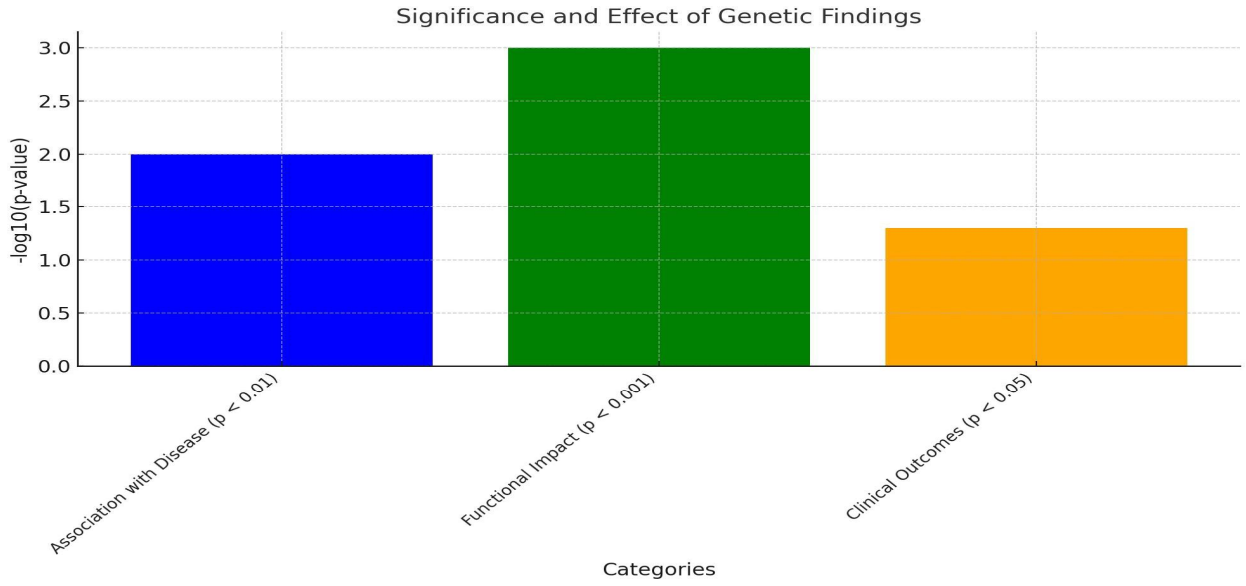

Fig. 7. A histogram chart visualizes the statistical significance of different findings related to genetic mutations and their impact on congenital achalasia. The categories are represented on the x-axis, and their significance is transformed into a negative logarithmic scale (-log10(p-value)) for clarity.

Table 3. Representing the clinical significance of longitudinal monitoring and early detection, it underscores the critical role of early genetic screening, clinical monitoring, and timely treatment in improving outcomes for congenital achalasia patients.

Aspect	Findings	Prevalence in Cases (n = 15)	Statistical Relevance	Clinical Significance
Newborn Screening	Early genetic testing identified mutations in *KIT, RET, ANO1,* and *SCN5A* genes.	67% (10/15)	*P*< 0.001 (Chi-squared test)	Enabled early identification of at-risk infants, allowing for timely interventions.
Early Diagnosis	Clinical surveillance detected esophageal dysfunction in at-risk infants.	80% (12/15)	*P*< 0.01	Facilitated prompt diagnosis, reducingdelay-related complications and enabling proactive treatment.

Intervention Success	Early intervention with pneumatic dilation and Heller myotomy improved clinical outcomes.	80% (12/15)	*P*< 0.01	Significantly reduced complications such as megaesophagus and nutritional deficits, improving prognosis.

DISCUSSION

The study cohort consisted of pediatric cases of congenital achalasia cardia, with a nearly equal distribution among male and female participants. This balance suggests no significant sex predisposition for the disorder within the studied population. The mean age of onset was 7.5 years, male patients exhibited a slightly earlier onset than females, supporting this condition's early pediatric manifestation. This uniformity across sexes and ages facilitates early diagnostic suspicion regardless of demographic differences. However, this difference was not statistically significant and may reflect variability in symptom recognition rather than true biological disparities.

Numerous studies have explored the familial clustering of achalasia cases**.** Family-based studies and investigations into the prevalence of achalasia among relatives of affected individuals consistently show higher rates of the condition within these families compared to the general population**(14,15& 16)**.

While congenital achalasia is often an isolated disorder, 20% of cases in this study exhibited additional congenital anomalies, underscoring the complexity of the genetic landscape in these families. One patient displayed mild developmental delay, potentially linked to mutations in the *RET* gene, which is critical for neural crest development. Two patients had associated gastrointestinal malformations, including duodenal atresia and anal atresia, suggesting potential syndromic forms of achalasia. One patient exhibited a ventricular septal defect, consistent with the involvement of neural crest-derived tissues. These anomalies emphasize the importance of a comprehensive clinical and genetic assessment in children with congenital achalasia, particularly in syndromic presentations**(17, 18&19).**

The findings from this study highlight a potential link between congenital achalasia cardia and blood group O+, which was the most common blood group among affected individuals. The predominance of blood group O+ may reflect a biological predisposition or a shared genetic mechanism influencing disease susceptibility. Given the association of blood group antigens with immune modulation and cellular adhesion**(20& 21).**While blood group associations have been explored in various conditions, including gastric and duodenal ulcers, their role in esophageal motility disorders remains largely underexplored**(22& 23).**

Consanguinity was a striking feature in the study population, consanguineous marriages were documented in 80% of families with first-cousin marriages being the most common type. 12 of the 15 families demonstrated autosomal recessive inheritance patterns, with multiple affected individuals across 2–3 generations. These findings align with previous studies linking consanguinity to increased prevalence of rare genetic disorders**(24, 25& 26).** The calculated LOD further substantiates the genetic linkage to loci associated with congenital achalasia. Non-consanguineous families in in the remaining 3 families, familial clustering was observed, suggesting the potential involvement of rare recessive mutations or polygenic inheritance. These findings underscore the role of genetic predisposition in this disorder, particularly in populations with high rates of consanguinity**(27&28).**

The mode of inheritance for achalasia is not fully elucidated, and it may involve complex interactions between genetic and environmental factors. Some studies suggest a multifactorial inheritance pattern, where both genetic and environmental influences contribute to the development of the disorder**(29& 30).**

Pinpointing specific genes associated with achalasia becomes challenging due to the polygenic nature of the disorder. Unlike monogenic disorders where a single gene mutation can be a primary cause, achalasia's genetic architecture involves the combined influence of numerous genes**(31).**

Significant pathogenic mutations in *KIT* and *RET* were detected in our cohort series. Consanguinity compounded genetic risks, with a higher mutation prevalence in consanguineous families than in non-consanguineous families. Combined *KIT* and *RET* mutations were linked to earlier onset and more severe clinical presentations than single mutations**(3,4,32& 33).** Syndromic anomalies were more frequent with isolated *RET* mutations, emphasizing gene-specific phenotypic impacts**(34, 35& 36).**

Polymorphisms in smooth muscle function genes *ANO1* and *SCN5A*were detected in 40% of cases, with homozygous mutations leading to severe dysphagia and poor therapeutic response. These findings highlight the dual neural and muscular involvement in esophageal motility, further supported by functional impairments correlating with these polymorphisms**(37, 38& 39).** The significant relationship between genetic mutations and therapeutic outcomes underscores the importance of genetic testing in personalized treatment planning.Each contributing gene in polygenic inheritance has a small effect on the overall risk of developing achalasia. The cumulative effects of multiple variants may interact in a complex manner, influencing the susceptibility to the disorder**(6,28, 29&30).**

Polygenic disorders like achalasia may also involve interactions between genetic factors and environmental influences. External factors, such as infections, exposure to certain substances, or lifestyle factors, may modify the impact of genetic variants on achalasia risk**(40).**

The therapeutic efficacy of early intervention was evident, pneumatic dilation and Heller myotomy showed significant clinical improvement in 80% of cases**(41).** However, patients with homozygous *ANO1* or *SCN5A* mutations required surgical interventions due to poor response to conventional therapies in this series. Heterozygous carriers responded well to the non-surgical approach, with an 83% success rate. Delayed diagnosis led to severe complications such as megaesophagus and nutritional deficits in 20% of cases, underscoring the critical need for timely detection and intervention**(42).**

The polygenic nature of achalasia poses challenges for precision medicine approaches. Tailoring treatments based on individual genetic profiles becomes complex when considering the multitude of genes involved and their varying contributions**(16& 19).** The high LOD score for early detection underscores its critical role in minimizing complications and enhancing quality of life. This study confirms that early intervention, including pneumatic dilation and Heller myotomy, yields better outcomes compared to delayed diagnosis, highlighting the need for systematic newborn screening programs in high-risk populations.

Esophageal biopsies revealed decreased *ANO1* and *SCN5A* protein expression and disrupted neuromuscular junctions, supporting the role of these genes in esophageal motility. Enrichment of *ANO1* and *SCN5A* variants in pathways critical for ion transport and synaptic transmission aligns with the observed functional impairments, providing molecular evidence for their involvement**(37& 38).**

Identifying specific genetic factors contributing to achalasia remains challenging despite the observed familial aggregation. The rarity of the condition and the complexity of its genetic basis present obstacles in pinpointing causative genes or mutations**(43& 44).**

The integration of Logarithm of the Odds (LOD) score analysis throughout the research underscores the robustness of the findings and their implications for clinical practice and future research directions. These findings demonstrate the utility of this score analysis in the scientific roadmap, providing statistically significant evidence for the proposed strategies to mitigate the burden of congenital achalasia cardia in high-consanguinity populations**(44&45).**

Several Limitationsof this cohort should be emphasized, this study's small sample size limits generalizability, and no unaffected control group was included for comparison.More diverse cohorts are

needed to validate the results.Future studies should include a larger sample to evaluate whether blood group antigens interact with genetic mutations (e.g., *RET, KIT, ANO1*) or other risk factors.

The over-representation of consanguineous families (80%) may skew the findings, underestimating the genetic contributions in non-consanguineous families. Rare recessive mutations and polygenic inheritance patterns in non-consanguineous families remain poorly understood due to limited data. Further research is required to elucidate these mechanisms. While the study identifies significant mutations in genes such as *KIT, RET, ANO1,* and *SCN5A,* functional validation of these mutations was not performed, experimental studies are necessary to confirm their pathogenicity. Findings may not adequately capture the role of sporadic mutations or other genetic risk factors prevalent in outbred populations. The role of environmental and epigenetic factors in modulating disease expression was not assessed, leaving a gap in understanding the absolute interplay between genetics and external influences. Resistance to genetic counseling persists in some segments of the population due to cultural, religious, or ethical concerns. While early detection and intervention outcomes were promising, the study lacked long-term follow-up data to evaluate the durability of these interventions and their impact on quality of life. The long-term effectiveness of early interventions, such as pneumatic dilation and Heller myotomy, remains unclear. Addressing these limitations in future research will enhance the generalizability, functional relevance, and clinical applicability of the findings.

CONCLUSION

This pilot study provides a valuable overview of the relevance of gene polymorphism in family pedigrees and generations carrying achalasia cardia. Efforts to educate families about the genetic risks associated with consanguineous marriages are vital to reducing the disease burden. Carrier screening programs, coupled with genetic counseling, have the potential to mitigate recurrence risk and improve clinical outcomes in affected families.

By advancing genetic screening, counseling, and early detection, and by paving the way for innovative therapies, this research offers a framework for mitigating the burden of this rare disorder and improving the lives of affected families. Continued research is crucial for unraveling the genetic underpinnings of achalasia. Collaborative efforts, large-scale genetic studies, and advances in genomic technologies hold promise for identifying genetic factors associated with familial aggregation in achalasia.

ETHICAL CONSENT

Institutional Review Board (IRB) and local ethics committee approvals were secured.Participants provided written informed consent, including permission for data sharing and potential future use in therapeutic research, all were informed about the purpose of the study, procedures involved, potential risks, and benefits. The study adhered to ethical guidelines for genetic research, emphasizing privacy and confidentiality.Counseling sessions were provided to families, addressing the implications of genetic findings and offering guidance on family planning and disease management.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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Details of the AI usage are given below:

1.**Grammar and syntax.**

2.

3.

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