Case Report

Unveiling a *TGFBI* Variant in the Retinal Capillary Hemangioblastoma, Type II Granular Corneal Dystrophy, and Von Hippel–Lindau Families: Unlocking Potential for Early Intervention and Targeted Therapy

Fatemeh Azimi[1], Golnaz Khakpour[1,2], Ahad Sedaghat[1], Fatemeh Mostafaiee[2], Hengameh Kasraei[1], Masood Naseripour[1,3]

[1]Eye Research Center, The Five Senses Institute, Iran University of Medical Sciences, Tehran, Iran, [2]Department of Medical Genetics, School of Medicine, Iran University of Medical Sciences, Tehran, Iran, [3]Finetech in Medicine Research Center, Iran University of Medical Sciences, Tehran, Iran

Abstract

Purpose: To identify the potential genetic factors responsible for retinal capillary hemangioblastoma (RCH) and Type II granular corneal dystrophy (GCDII), with autosomal dominant inheritance. We used whole-exome sequencing (WES) in an Iranian family to identify the possible genetic etiology of RCH and GCDII with other manifestations of von Hippel–Lindau (VHL) disease.

Methods: This study included one Iranian family for WES in index patients and Sanger sequencing in all available individuals.

Results: Clinical presentations of these patients included RCH, GCD, central nervous system hemangioblastoma as well as pancreatic cyst. WES disclosed a heterozygous known pathogenic variant c.371G>A (p.R124H) in exon 4 of gene *TGFBI*.

Conclusions: For the first time, our research identified the potential involvement of *TGFBI*: c.371G>A (p.R124H) in an Iranian family with RCH, GCDII, and other symptoms of VHL disease. In the future, *TGFBI* could offer a new understanding and a promising therapeutic approach for both GCDII and VHL diseases simultaneously. Before using the variant in genetic counseling, it is recommended to conduct functional analysis using appropriate animal models to understand its pathogenesis mechanism.

Keywords: Granular corneal dystrophy, Retinal capillary hemangioblastoma, Von Hippel–Lindau, Whole-exome sequencing

Address for correspondence: Masood Naseripour, Rassoul Akram Hospital, Niayesh Ave., Sattarkhan St. Tehran 14455-364, Iran.
E-mail: masoodnp@yahoo.com
Submitted: 01-Mar-2024; **Revised:** 25-Jun-2024; **Accepted:**25-Jun-2024; **Published:** 18-Jan-2025

Introduction

Von Hippel–Lindau (VHL) syndrome is an autosomal dominant hereditary neoplastic disorder characterized by the central nervous system hemangioblastoma (CNS-HB) and retinal capillary hemangioblastoma (RCH), renal cell carcinoma (RCC), pheochromocytoma, and other multiple tumors or cysts of the kidney, liver, pancreas, and epididymis.[1,2] RCH is a hallmark lesion that often presents at an early stage in the VHL disease,[3] which is caused by mutations in the VHL tumor-suppressor gene, located on chromosome 3p25–26.[1,2] A component of a ubiquitination complex is encoded by this gene.

The encoded protein bonds hydroxylated target proteins to oxygen-bound proteins, leading to polyubiquitination and degradation. The transcription factor hypoxia-inducible factors 1α (HIF1α) and HIF2α are the most characterized VHL substrates.[4] VHL deficiency results in the stabilization of HIFα (1 and 2), leading to the transcriptional activation of HIF

Access this article online

Quick Response Code:

Website: https://journals.lww.com/joco

DOI: 10.4103/joco.joco_53_24

How to cite this article: Azimi F, Khakpour G, Sedaghat A, Mostafaiee F, Kasraei H, Naseripour M. Unveiling a *TGFBI* variant in the retinal capillary hemangioblastoma, type II granular corneal dystrophy, and von Hippel–Lindau families: Unlocking potential for early intervention and targeted therapy. J Curr Ophthalmol 2024;36:205-9.

targets that promote angiogenesis (e.g., vascular endothelial growth factor A and platelet-derived growth factor), metabolic reprogramming toward the Warburg effect (e.g., glucose transporter 1, hexokinase 2, and lactate dehydrogenase A), cell proliferation (e.g., transforming growth factor α, EGFR, and NF-κB), and other malignancy-associated features.[4-6]

In this study, we aim to describe a family with Type II granular corneal dystrophy (GCDII) and other manifestations of VHL disease.

Case Report

A 40-year-old man presented with blurred vision in 2003. He was identified as the proband (III-1). His pulse rate and blood pressure were both 100/min and 116/80 mmHg in the first examination. The diagnosis of VHL disease in family members was made through the ophthalmic examination and systemic screening tests. If patients have either one of the diagnostic criteria present in a first-degree family member or two VHL-related manifestations without a known family history of VHL, they can be diagnosed as VHL cases. The clinical phenotype of GCDII was confirmed by slit-lamp examination and *in vivo* confocal microscopy (IVCM) of diffuse linear, stellate opacities, and granular.

Fundoscopy revealed bilateral RCH (the left eye with multiple RCH and the right eye with single RCH). Fluorescein angiographic findings were compatible with a diagnosis of RCHs in both eyes [Figure 1]. Slit-lamp examination and IVCM showed bilateral diffuse linear, granular, and stellate opacities were suggestive of a clinical phenotype of GCDII. The best-corrected visual acuity (BCVA) of his right eye and left eye were 10/10 and 6/10, respectively. Intraocular pressure (IOP) was 10 mmHg in both eyes. Both globes of the patients were preserved during the follow-up period. Using computed tomography and magnetic resonance imaging (MRI), pathological lesions were detected in the brain (CNS-HB) and other organs (pancreatic cyst [PC]). His parents were healthy, with normal vision, and were not consanguineous in marriage. His other family members have CNS-HB signs (II-11, 12 and 13 and III-4 and 8). The pedigree of the studied family is shown in Figure 2.

His son was diagnosed with unilateral single RCH in his left eye at the age of 8 (IV-1) and presented with blurred vision in 2022. At the first visit, his pulse rate and blood pressure were 100/min and 100/80 mmHg, respectively. The BCVA of his left eye was 8/10, and his IOPs were 13 mmHg in the left eye.

His sister (III-2) who was 38 years old presented with blurry vision in 2005. She presented symptoms such as proband (III-1) (RCH, GCD, CNS-HB, and PC). Her pulse rate and blood pressure were 100/min and 150/85 mmHg accordingly during the first examination. Fundoscopy revealed unilateral RCH in her left eye. The BCVA of her left eye was 3/10, and her IOPs were measured at 14 mmHg. MRI revealed pathological lesions in the brain and pancreas.

His niece (IV-3) developed bilateral RCHs at the age of 15. The BCVA of both eyes was 10/10, and the IOP was 14 mmHg in both eyes. Like her mother, the daughter's corneas at slit-lamp examination showed bilateral diffuse linear, stellate, and granular opacities.

The Ethics Committee of the Iran University of Medical Sciences in Tehran, Iran, has approved the experimental protocol of this study with an approval number of IR.IUMS.REC.1396.32903. All of the patient's clinical information and medical histories were collected at the Rasoul- Akram Hospital, Tehran, Iran. Genetic counseling was given to the entire family, and informed consent was obtained from the parents or guardians of all patients who participated in the study.

Three milliliter of blood samples was collected from all available individuals in ethylenediaminetetraacetic acid anticoagulant tubes. DNA was extracted using the PrimePrep Genomic DNA Extraction Kit (Genet Bio, Korea) following standard protocol. The spectrophotometric (NanoDrop 2000, Thermo Fisher Scientific, US) measurement of 260 nm and 280 nm wavelengths was used to measure the quality and quantity of extracted DNA. A ratio of 1.7–2.0 is generally accepted as "pure" for DNA.

Whole-exome sequencing (WES) was performed on isolated DNA in index patients. In the Macrogen Korea sequencing order system (Macrogen Inc., Seoul, South Korea), 3 μg of gDNA samples was subjected to WES. A paired-end, high-throughput sequencing reads of 101 bp was carried out with Illumina (Illumine Inc., San Diego, CA, USA) using the HiSeq 4000 Sequencing Platforms based on an Agilent Sure Select Target V6-Postenrichment Kit preparation guide from Agilent Technologies, Inc., Santa Clara, CA, USA.

Using Fast QC software, the FASTQ files were initially assessed. The BWA algorithm (bwa-0.7.12) was employed for mapping the reference genome (GRCh37/hg19).[7] Picard (Picard-tools-1.130) served as the tool we utilized for identifying duplicates. We employed HaplotypeCaller in GATK (GATKv3.4.0) for indels realignment, base calibration, variant calling, and filtering. In the end, both SnpEff (SnpEff_v4.1g)[8] were utilized for variant annotation (wannovar.wglab.org).[9]

Variants with minor allele frequency less than 0.01, population frequency, dbSNP (ncbi.nlm.nih.gov/snp), ExAC (exac.broadinstitute.org), 1000GP (internationalgenome.org), and ESP6500 (evs.gs.washington.edu/EVS) are present in the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/), and ESP6500 (evs.gs.washington.edu/EVS) were selected for further analysis. Intronic and intergenic variants were eliminated through a location-based filtering process. Combined Annotation Dependent Depletion (CADD) was utilized to assess the potential impact of giving variants. MutationTaster (mutat iontaster.org)[10] examined variants with a CADD-Phred score (cut-off >10) for disease-causing effects and then used Franklin by Genoox and Varsome tools to interpret and categorize it according to the ACMG variant classification guideline.[11] The role of candidate genes in VHL with GCDII was reviewed through various databases (GWAS catalog, OMIM, etc.).[12,13]

To confirm the WES findings, family members were subjected to reverse Sanger sequencing on exon 4 of the *TGFBI* gene. Primers used for Sanger sequencing were designed using NCBI Primer-BLAST software. Primer sequences were F: 5'TCCCTCCTTCTGTCTTCTGC 3' and R: 5'CTCGGGGAAGTAAGGCAGTT 3'. The ABI 3730 sequencer (Bioneer, South Korea) was used for sequencing using the Sanger method.

We studied several affected and asymptomatic proband's mothers of a pedigree, aged 5–40 years. A history of visual problems was found in four individuals who appeared to be closely related. The affected children showed an RCH phenotype and a range of VHL disease symptoms. However, GCD was seen in their parents' generation.

To identify the gene responsible for the onset of RCH and GCDII with other manifestations of VHL disease in a family, WES was performed in proband diagnosed with VHL disease.

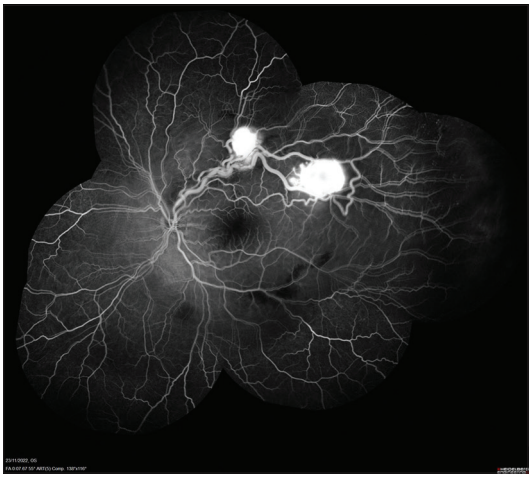

Figure 1: Fluorescein angiographic. Left eye fluorescein angiography revealed multiple retinal capillary hemangioblastoma with feeder and drainer vessels

The disruptive effect of the variant was revealed by *in silico* prediction tools and additional studies [Table 1].

WES revealed a known missense disease-causing variant, c.371G>A (p.R124H), within exon 4 of the *TGFBI* gene. It was confirmed by Sanger sequencing on affected individuals and the asymptomatic proband's mother [Figure 3].

Based on ACMG classification, the c.371G>A variant in the *TGFBI* gene is pathogenic. In addition, multiple lines of computational evidence such as BayesDel_addAF, DANN, DEOGEN2, Eigen, FATHMM-MKL, LIST-S2, M-CAP, MVP, Mutation Assessor, MutationTaster, and SIFT support a deleterious effect of this mutation on the gene or gene product.

Discussion

Herein, we conducted WES on proband with RCH, GCDII, and other manifestations of VHL disease. WES was performed on index patients and Sanger sequencing in all available individuals. For the first time, we identified a pathogenic variant of the *TGFBI* gene that was responsible for the VHL and GCDII in the family, expanding the *TGFBI* gene's mutational spectrum.

In this study, a heterozygous variant of the *TGFBI* gene was detected in all six affected and one unaffected participant, with variant c.371G>A (p.R124H). The origin of this disease was traced back to the mother's family. Due to reduced penetrance or variable expressivity, the mother of the proband might not have shown any signs of the disease; however, she carried the mutated gene that could be passed on to future generations.

TGFBI (OMIM 601692, GRCh38:CM000667.2, previously called *BIGH3*) gene was discovered in 1992 on chromosome 5q31.1 and has 17 exons, which total approximately 35 kb.

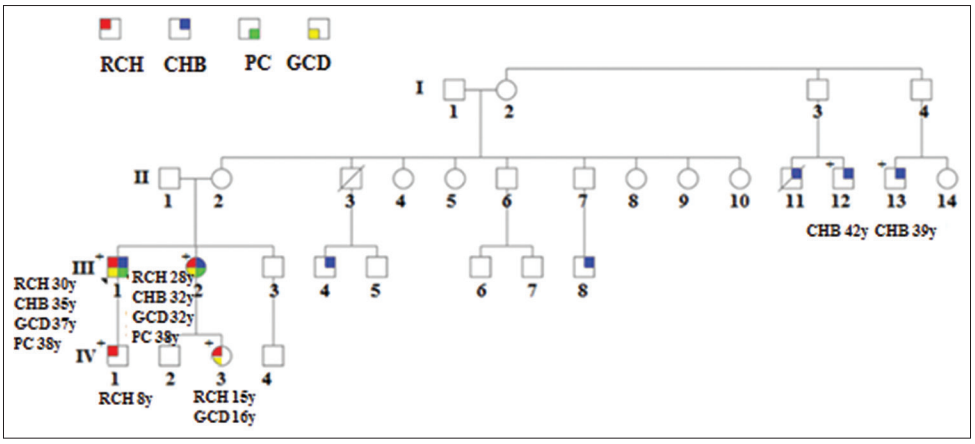

Figure 2: Pedigree of the affected family. Color symbols represent the patients. The proband is indicated with arrows. The affected subjects II-11, 12, and 13 and III-4 and 8 manifested central nervous system hemangioblastoma (CNS-HB). The proband III-1 and his sister (III-2) had retinal capillary hemangioblastoma (RCH), CNS-HB, granular corneal dystrophy (GCD), and pancreatic cyst. The affected subject IV-1 had RCH and IV-3 represented RCH and GCDII. The heterozygous missense mutation, c.371G>A (p.R124H), in the exon 4 of the transforming growth factor beta-induced gene was detected in this case. A positive sign (+) is placed in those individuals with found variants. RCH: Retinal capillary hemangioblastoma, GCD: Granular corneal dystrophy, PC: Pancreatic cyst

The *TGFBI* protein in humans is made up of 683 amino acids and has a predicted molecular weight mass of 68 kDa. The N-terminus contains a secretory signal peptide followed by a cysteine-rich EMI domain. The core of *TGFBI* contains four fasciclin-1 (FAS1) domains, and at the C-terminus, there is a single arginine–glycine–aspartic sequence. The variant may be responsible for altering protein solubility and stability by altering the first FAS1 domain of the protein [Figure 4].[14]

Many studies have reported that corneal dystrophies are commonly diagnosed due to autosomal dominant missense mutations in the *TGFBI* gene. This gene encodes an extracellular matrix protein that is believed to play important roles in physiologic and pathologic responses by regulating cell adhesion, proliferation, migration, and differentiation.[15] GCDII is multiple small deposits in the superficial central corneal stroma. This rare form of stromal corneal dystrophy can occasionally result in significant visual impairment.[16]

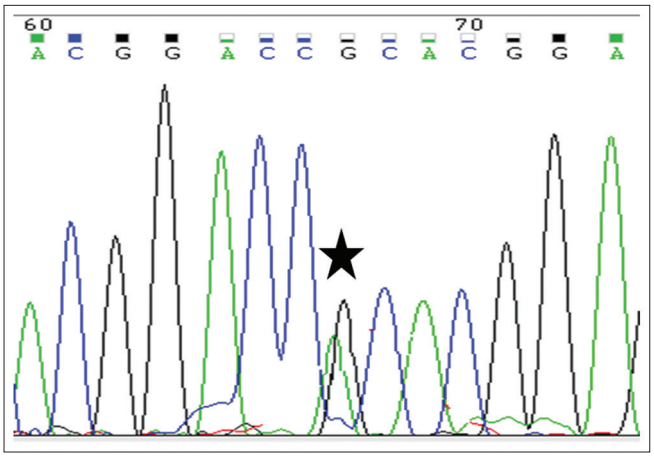

Figure 3: DNA sequences for pathogenic variant c.371G>A (p.R124H) in exon 4. Heterozygous state for the variant c.371G>A, G and A peaks are visible by star

Several studies have reported that VHL represses *TGFBI* expression independent of HIF, and loss of VHL leads to increased *TGFBI* levels, indicating that *TGFBI* is a direct target of VHL.[17,18] Moreover, it is recognized that *TGFBI* acts a crucial role for the promotion of bone metastasis in clear cell RCC by suppressing osteoblast differentiation.[19] The presence of the *TGFBI* protein overexpression is significantly correlated with advanced tumor stage, metastasis, and cancer-specific mortality in clear cell RCC patients.[20]

For the first time, our research revealed the potential significance of *TGFBI*: c.371G>A (p.R124H) in an Iranian family with RCH, GCDII, and VHL disease. In familial cases, WES should be performed as the initial molecular diagnostic test as we recommend. *TGFBI* may provide new insight and a promising therapeutic approach for both GCDII and VHL diseases simultaneously in the future. Before using genetic counseling, conducting a functional analysis using appropriate animal models is advisable to understand the pathogenesis mechanism of the variant.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given his consent for his images and other clinical information to be reported in the journal. The patient understands that his name and initials will not be published and due efforts will be made to conceal his identity, but anonymity cannot be guaranteed.

Acknowledgments

The authors thank all those who provided support during this research.

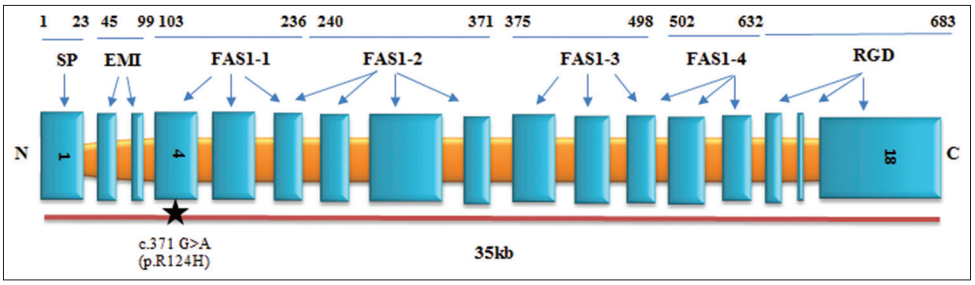

Figure 4: The TGFBI gene and protein structure are represented graphically, with the position of the detected mutation exon and the associated domain visible. The boundaries between exons and introns are visible in scale as exons are drawn to scale and introns are not drawn to scale. *Indicate where the mutation occurred. Transcript NM_000358. FAS1: Fasciclin-1, RGD: Arginine–glycine–aspartic

Table 1: *In silico* analysis of the variant pathogenicity for c.371G>A in *TGFBI*

Gene	Position/variant	Zygosity	Inheritance	Disease	MAF*/RS	ACMG classification
TGFBI	Chr5:135382096:G>A:: NM_000358.3:exon4:c. 371G>A: p.R124H	HET	Autosomal dominant	CDGGII	0.0000402/rs121909211	Pathogenic

*MAF was extracted from gnomAD database. gnomAD: Genome aggregation database, HET: Heterozygous, CDGGII: Corneal dystrophy, groenouw type II, MAF: Minor allele frequency

Financial support and sponsorship

This work is supported by Deputy for Research of Iran University of Medical Sciences (IUMS) and IUMS Eye Research Center, Tehran, Iran.

Conflicts of interest

There are no conflicts of interest.

References

1. Chittiboina P, Lonser RR. Von Hippel-Lindau disease. Handb Clin Neurol 2015;132:139-56.
2. Maher ER, Kaelin WG Jr. Von Hippel-Lindau disease. Medicine (Baltimore) 1997;76:381-91.
3. Wong WT, Agrón E, Coleman HR, Tran T, Reed GF, Csaky K, *et al.* Clinical characterization of retinal capillary hemangioblastomas in a large population of patients with von Hippel-Lindau disease. Ophthalmology 2008;115:181-8.
4. Gossage L, Eisen T, Maher ER. VHL, the story of a tumour suppressor gene. Nat Rev Cancer 2015;15:55-64.
5. Keith B, Johnson RS, Simon MC. HIF1α and HIF2α: Sibling rivalry in hypoxic tumour growth and progression. Nat Rev Cancer 2011;12:9-22.
6. Lu X, Kang Y. Hypoxia and hypoxia-inducible factors: Master regulators of metastasis. Clin Cancer Res 2010;16:5928-35.
7. Li H. Exploring single-sample SNP and INDEL calling with whole-genome *de novo* assembly. Bioinformatics 2012;28:1838-44.
8. Cingolani P, Platts A, Wang le L, Coon M, Nguyen T, Wang L, *et al*.. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin) 2012;6:80-92.
9. Yang H, Wang K. Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR. Nat Protoc 2015;10:1556-66.
10. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods 2010;7:575-6.
11. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, *et al.* Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17:405-24.
12. MacArthur J, Bowler E, Cerezo M, Gil L, Hall P, Hastings E, *et al.* The new NHGRI-EBI catalog of published genome-wide association studies (GWAS Catalog). Nucleic Acids Res 2017;45:D896-901.
13. Hamosh A, Scott AF, Amberger JS, Bocchini CA, McKusick VA. Online Mendelian Inheritance in Man (OMIM), a knowledgebase of human genes and genetic disorders. Nucleic Acids Res 2005;33:D514-7.
14. Skonier J, Neubauer M, Madisen L, Bennett K, Plowman GD, Purchio AF. cDNA cloning and sequence analysis of beta ig-h3, a novel gene induced in a human adenocarcinoma cell line after treatment with transforming growth factor-beta. DNA Cell Biol 1992;11:511-22.
15. Han KE, Choi SI, Kim TI, Maeng YS, Stulting RD, Ji YW, *et al.* Pathogenesis and treatments of TGFBI corneal dystrophies. Prog Retin Eye Res 2016;50:67-88.
16. Evans CJ, Davidson AE, Carnt N, Rojas López KE, Veli N, Thaung CM, *et al.* Genotype-phenotype correlation for TGFBI corneal dystrophies identifies p.(G623D) as a novel cause of epithelial basement membrane dystrophy. Invest Ophthalmol Vis Sci 2016;57:5407-14.
17. Shang D, Liu Y, Yang P, Chen Y, Tian Y. TGFBI-promoted adhesion, migration and invasion of human renal cell carcinoma depends on inactivation of von Hippel-Lindau tumor suppressor. Urology 2012;79:966.e1-7.
18. Ivanov SV, Ivanova AV, Salnikow K, Timofeeva O, Subramaniam M, Lerman MI. Two novel VHL targets, TGFBI (BIGH3) and its transactivator KLF10, are up-regulated in renal clear cell carcinoma and other tumors. Biochem Biophys Res Commun 2008;370:536-40.
19. Pan T, Lin SC, Yu KJ, Yu G, Song JH, Lewis VO, *et al.* BIGH3 promotes osteolytic lesions in renal cell carcinoma bone metastasis by inhibiting osteoblast differentiation. Neoplasia 2018;20:32-43.
20. Lebdai S, Verhoest G, Parikh H, Jacquet SF, Bensalah K, Chautard D, *et al.* Identification and validation of TGFBI as a promising prognosis marker of clear cell renal cell carcinoma. Urol Oncol 2015;33:69.e11-8.