Int J Clin Exp Pathol 2017;10(8):8709-8714
www.ijcep.com /ISSN:1936-2625/IJCEP0058353

Original Article

Long-term follow-up of an Alport syndrome patient with a novel mutation of *COL4A5*

Rong Xiang[1], Jing-Jing Li[1], Ji-Shi Liu[2], Liang-Liang Fan[1], Lin Li[2], Kun Xia[1], Hao Zhang[2]

[1]*The State Key Laboratory of Medical Genetics & School of Life Sciences, Central South University, Changsha, China;* [2]*Department of Nephrology, The Third Xiangya Hospital of Central South University, Changsha, China*

Received May 27, 2017; Accepted May 27, 2017; Epub August 1, 2017; Published August 15, 2017

Abstract: Background: Alport syndrome (AS) is a genetic disease characterized by progressive glomerulonephritis with a high life-time risk for end-stage renal disease (ESRD), sensorineural hearing loss and ocular abnormalities. So far, a lot of mutations were reported in *COL4A3, COL4A4* and *COL4A5* genes, which are related to AS. Methods: Whole-exome sequencing in combination with AS-related genes filtering strategy was applied to investigate a Chinese AS family. We also employed Sanger sequencing to confirm the family co-segregation. In addition, we also summed up a long-term follow-up data from 2003 to 2016. Results: In this study, we have detected a novel insertion mutation (c.348_349insTCCGG/p.G117Sfs×40) of *COL4A5*, which may lead to a truncated protein in the proband. Sanger sequencing confirmed that this novel mutation was co-segregated with all the family members. The long-term follow-up data showed that the progress of chronic kidney disease become more and more serious in the proband. Conclusions: A novel mutation (c.348_349insTCCGG/p.G117Sfs×40) of *COL4A5* was identified in this study. In addition, approximately 15 years long-term follow-up data was provided in this paper. Our study not only expands the spectrum of *COL4A5* mutations, but also analysis the progress of AS and fills the knowledge about course and potential further complications and health risks of AS.

Keywords: Alport syndrome, *COL4A5*, insertion mutation, long-term follow-up, whole-exome sequencing

Introduction

Alport syndrome (AS, OMIM: 301050) is characterized by proteinuria, hematuria, progressive glomerulonephritis with a high life-time risk for end-stage renal disease (ESRD), sensorineural hearing loss and ocular abnormalities [1-4]. In addition, several ophthalmic complications may also appear in this disorder. The estimated prevalence of this disorder is more than 0.01%.

Previous study have demonstrated collagen IV α3/α4/α5 network from the basement membranes of the glomerular, lens capsule, cornea and retina are related to AS [5]. Mutations in the *COL4A3* (NM_000091), *COL4A4* (NM_000092) and *COL4A5* (NM_033380) genes may break the stability of collagen IV α3/α4/α5 network and lead to AS [6-8]. However, due to genetic heterogeneity, high cost of testing technique and lacking awareness of testing, there are still a lot of AS patients lacking genetic testing and long-term follow-up records.

In this research, we investigate a family diagnosed as AS. An obvious X-dominant inheritance has been observed in this family. By using whole-exome sequencing, a novel insertion mutation (c.348_349insTCCGG/p.G117SfsX40) of *COL4A5* in X chromosome was identified. We also collected and summed up a follow-up clinic data of the proband from 2003 to 2016, this data may help us know more about the progress of this disease.

Subjects and methods

Patients and subjects

This research has been approved by Third Xiangya Hospital of the Central South University. All subjects have consented to this study. In this study, a family with 15 members across four

Table 1. Summary of a family with Alport syndrome

Family member	Disease	Sex	Age	COL4A5		Prediction by programs		
				DNA	Protein	Polyphen2	SIFT	MutationTaster
III2	Alport syndrome	Male	34 y	c.348_349insTCCGG	p.G117Sfs×40	Probably damaging	Deleterious	Disease causing
I1	Mild hematuria	Female	-	-	-			
I2	NO	Male	79 y	-	-			
II1	NO	Male	54 y	-	-			
II2	Mild hematuria	Female	55 y	c.348_349insTCCGG	p.G117Sfs×40	Probably damaging	Deleterious	Disease causing
II3	Alport syndrome	Male	58 y	c.348_349insTCCGG	p.G117Sfs×40	Probably damaging	Deleterious	Disease causing
II4	NO	Female	60 y	-	-			
III1	NO	Female	30 y	-	-			
III3	Uremia	Male	-					
III4	NO	Male	40 y	-	-			
III5	Mild hematuria	Female	39 y	c.348_349insTCCGG	p.G117Sfs×40	Probably damaging	Deleterious	Disease causing
IV1	Mild hematuria	Female	7 y	c.348_349insTCCGG	p.G117Sfs×40	Probably damaging	Deleterious	Ddisease causing
IV2	NO	Male	6 y	-	-			
IV3	Alport syndrome	Male	19 y	c.348_349insTCCGG	p.G117Sfs×40	Probably damaging	Deleterious	Disease causing
IV4	NO	Female	15 y	-	-			

generations from Hunan Province was investigated. The proband and seven family members were diagnosed with AS (**Table 1**).

Whole-exome sequencing

Genomic DNA was extracted with a DNeasy blood and tissue kit (Qiagen, Valencia, Calif., USA) [9]. The Novogene Bioinformatics Institute (Beijing, China) provided the exome capture, high throughput sequencing and common filtering. All the exomes were captured by means of Agilent SureSelect Human All Exon V5 kits and were sequenced with an Illumina HiSeq X Ten platform. Filtering strategies referred to our previous study [10].

Co-segregation analysis

Segregation analysis was applied in all family members according to the whole-exome sequencing results. Primer pairs were designed by Primer 5 and the sequences of primers will be provided upon request.

Results

Clinical features

We described a Chinese family with AS (**Table 1**; **Figure 1A**). The proband (III2), a 34-year-old officer from the Central-South China (Changsha, Hunan), had a 13-year history of chronic kidney disease (CKD) and diagnosed as AS. His younger brother (III-3) died from uremia in 2008, the affected male members (II-3, IV-3) were all diagnosed as typical AS and some female members (I-1, II-2, III-5, IV-1) were detected with mild hematuria, no other symptoms were observed in these female members.

Genetic analysis

Data filtering excluded shared common variants present in YH database, ESP database, 1000 Genomes Project and dbSNP132. About 1127 unique single nucleotide polymorphisms (SNPs) were detected in this family. After AS-related genes filtering, only a novel insertion mutation (c.348_349insTCCGG/p.G117SfsX40) of *COL4A5* in X chromosome was identified in the proband. Sanger sequencing have confirmed that this novel mutation was co-segregated with all the family members. (**Figure 1B**), which indicates that this insertion mutation in *COL4A5* may underlie this inherited disorder. Cross-species alignment analysis of COL4A5 amino acid sequences revealed that this mutated site was highly evolutionarily conserved (**Figure 1C**).

Long-term follow-up data

All the follow-up data of the proband are shown in **Table 2**. The proband was first presented at our hospital with proteinuria and hematuria in 2003. In the past decades, he received the drug therapy by Uremic Clearance Granule, Jinshuibao capsule, Bailing Capsule and other ancillary drugs. The follow-up was also started at this time. The cataract and respiratory tract infection was appeared during this time (2003-2007). In 2007, he was diagnosed with AS. During 2007 to 2014, the patient received fol-

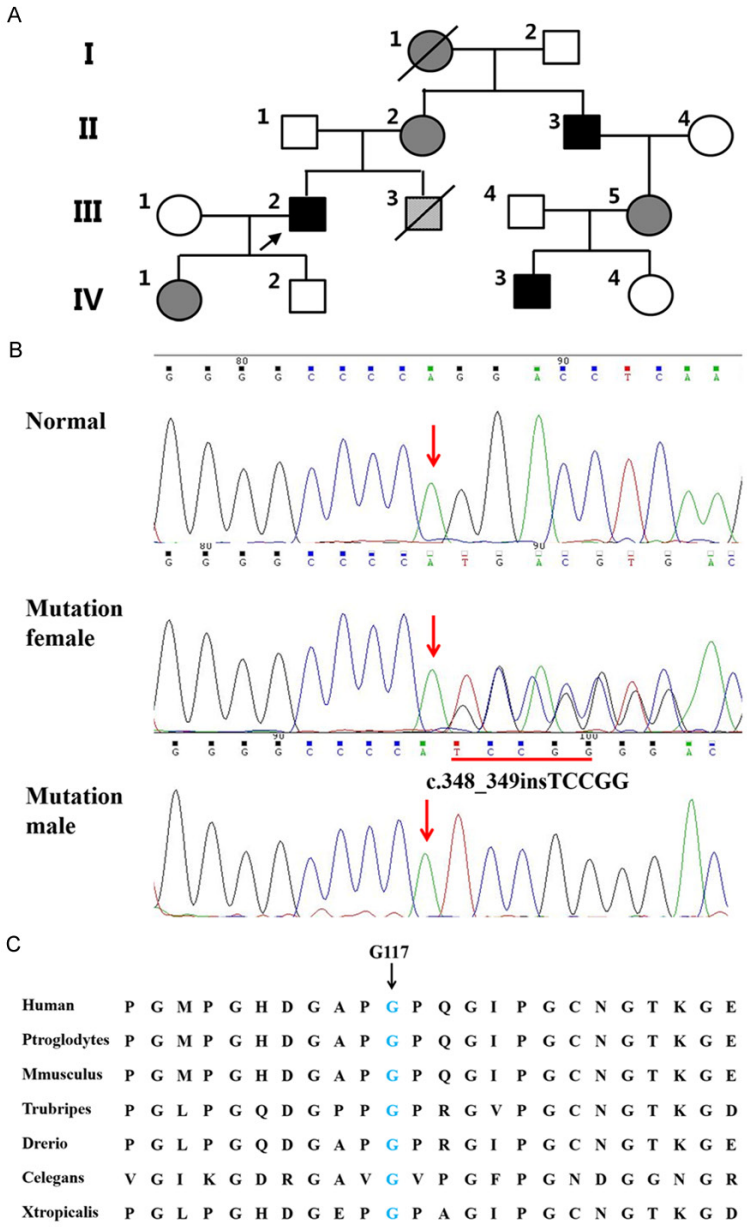

Figure 1. A. Pedigree involved in this study, there were 15 numbers totally. □, male members; ○, female members; ▨, deceased male member who was affected by uremia; ⌀, deceased affected female member with mild hematuria; ●, affected female members with mild hematuria, who has the insertion mutation (c.348_349insTCCGG/G117Sfs*40); ↗■, the proband; ■, affected male members who was diagnosed as typical Alport syndrome. B. Analysis of COL4A5. The chromatograms show partial sequence of *COL4A5*, c.348_349insTCCGG/G117Sfs*40, they are control, female patient and male patient. C. Conservation analysis of COL4A5. G117 sites are highlighted in blue. It is highly conserved at protein level across human, Ptroglodytes, Mmusculus, Trubripes, Drerio, Celegans, Xtropicalis, etc.

low-up visit 12 times and his symptom of the disease became more severe (from CKD3 to CKD5). In 2014, the proband received a kidney transplant but proteinuria symptom appeared again with an unexplained reason in 2016. Therefore, he has visited the hospital for further treatment recently.

Discussion

In this study, we applied whole-exome sequencing to identify the possible pathogenic genes for the family with AS. A novel insertion mutation (c.348_349insTCCGG/p.G117Sfs×40) in *COL4A5* was identified in this family. This insertion mutation locates in exon 6 of *COL4A5*, a highly conserved collagenous domain with 1430 residues, which leads to a truncated protein. Our genetic study confirmed the clinic diagnosis of AS.

COL4A5 gene contains 51 exons, encoding type IV collagen α5 chain which could combine with COL4A3 and COL4A4, forming triple-helical domains which located in glomerular basement membrane. Type IV collagen α5 chain contains 1,685 amino acid residues, including a 26-residue signal peptide, a 1,430-residue collagenous domain and a 229-residue carboxyl-terminal non-collagenous domain (NC1 domain) [11-13]. The 1,430-residue collagenous domain is the most significant domain, which plays a crucial role in the combination with COL4A3 and COL4A4. But this insertion mutation would interrupt its binding domain and affect triple-helical domains' function, finally, leading to AS [14].

Because *COL4A5* is too long to detect via Sanger sequencing, most of *COL4A5* mutations were identified by next-generation sequencing in recent years. *COL4A5* is located at Xq22.3 [7, 15]. Disease related to this gene shows a gender difference in severity with

Table 2. Long-term follow-up of the patients with Alport syndrome

Time/phenotype		2003.11	2006.2	2007.6	2007.11	2007.12.	2011.1	2011.7	2011.1	2012.3	2012.7
Kidney	Proteinuria	++++	+++	++	+++	++	++	++	+++	+++	+++
	Hematuria (RBC/mL)	100000	35000	25000	8500	15000	7500	40000	150000	2000	20000
Sensorineural deafness		-	-	-	-	-	-	-	-	-	-
Cataracts		-	-	-	-	√	√	√	√	√	√
Trachea		Pharynx swelling	-	Cough	Sore throat	-	-	-	-	-	Cough
Esophagus		Pharynx swelling	-	-	Sore throat	-	-	-	-	-	-
Anemia		-	-	-	-	-	-	-	-	-	-
Stages		-	-	-	CKD3	CKD3	CKD3	CKD3	CKD3	CKD4	CKD3
Alport syndrome		-	-	-	-	√	√	√	√	√	√
Kidney transplant		-	-	-	-	-	-	-	--		-
Time/phenotype		2012.12	2013.5	2013.1	2013.12	2014.5	2014.6	2014.8	2014.11	2015.4	2016.4
Kidney	Proteinuria	+++	+++	++	++	++	++	-	-	-	++
	Hematuria (RBC/mL)	65000	67500	10000	27500	12500	10000	-	-	<8000	<8000
Sensorineural deafness		-	-	-	-	-	-	-	-	-	-
Cataracts		√	√	√	√	√	√	√	√	√	√
Trachea		-	-	-	-	-	-	-	Cough	-	-
Esophagus		-	-	-	-	-	-	--	-	-	-
Anemia		√	√	-	√	√	√	√	-	-	-
Stages		CKD3	CKD3	CKD3	CKD3	CKD3	CKD4	CKD5	-	-	
Alport syndrome		√	√	√	√	√	√	√	√	√	√
Kidney transplant		-	-	-	-	-	-	√	-	-	-

males showing more severe symptoms. Only 12% female patients with X-linked AS reach end-stage renal disease before the age of 40 and only 30% before the age of 60 in European cohort [16, 17]. While 70% male patients with X-linked AS had end-stage renal disease before the age of 30 and the remaining 30% after their 30 [18, 19]. In our study, males with AS exhibited more severe symptoms than females, which is consistent with previous studies [16-19].

In this study, we also performed a long-term follow-up of an AS patient with *COL4A5* mutation. The clinic data from the first hospitalization with proteinuria and hematuria in 2003 to renal transplant in 2014. We can conclude that this disease was a progressive disease, especially the progress of chronic kidney disease. Our concluded data may contribute to fill the knowledge about course and potential further complications and health risks of AS.

To sum up, we identified a novel insertion mutation (c.348_349insTCCGG/p.G117Sfs×40) of *COL4A5* in X chromosome by whole-exome sequencing in a family with AS. And we performed a long-term follow-up for about 15 years of the proband. Our research not only expands the spectrum of *COL4A5* mutations, but also provides more knowledge about course and potential further complications and health risks during adulthood and advanced age.

Acknowledgements

We thank the patients and their families for participating in this study. This study was supported by the National Natural Science Foundation of China (81370394, 81600535), the National Natural Science Foundation of Hunan province (2016jj4106), and the Fundamental Research Funds for Central Universities of Central South University (2017zzts348).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hao Zhang, Department of Nephrology, The Third Xiangya Hospital of Central South University, Central South University, Changsha, China. E-mail: zhanghaoliaoqing@163.com

References

[1] Gross O, Kashtan CE, Rheault MN, Flinter F, Savige J, Miner JH, Torra R, Ars E, Deltas C, Savva I, Perin L, Renieri A, Ariani F, Mari F, Baigent C, Judge P, Knebelman B, Heidet L, Lagas S, Blatt D, Ding J, Zhang Y, Gale DP, Prunotto M, Xue Y, Schachter AD, Morton LC, Blem J, Huang M, Liu S, Vallee S, Renault D, Schifter J, Skelding J, Gear S, Friede T, Turner AN and Lennon R. Advances and unmet needs in genetic, basic and clinical science in Alport syndrome: report from the 2015 International workshop on Alport syndrome. Nephrol Dial Transplant 2017; 32: 916-924.

[2] Savva I, Pierides A and Deltas C. RAAS inhibition and the course of Alport syndrome. Pharmacol Res 2016; 107: 205-210.

[3] Kamiyoshi N, Nozu K, Fu XJ, Morisada N, Nozu Y, Ye MJ, Imafuku A, Miura K, Yamamura T, Minamikawa S, Shono A, Ninchoji T, Morioka I, Nakanishi K, Yoshikawa N, Kaito H and Iijima K. Genetic, clinical, and pathologic backgrounds of patients with autosomal dominant Alport syndrome. Clin J Am Soc Nephrol 2016; 11: 1441-9.

[4] Malone AF, Funk SD, Alhamad T and Miner JH. Functional assessment of a novel COL4A5 splice region variant and immunostaining of plucked hair follicles as an alternative method of diagnosis in X-linked Alport syndrome. Pediatr Nephrol 2017; 32: 997-1003.

[5] Wiersma AC, Millon LV, Hestand MS, Van Oost BA and Bannasch DL. Canine COL4A3 and COL4A4: sequencing, mapping and genomic organization. DNA Seq 2005; 16: 241-251.

[6] Savige J, Sheth S, Leys A, Nicholson A, Mack HG and Colville D. Ocular features in Alport syndrome: pathogenesis and clinical significance. Clin J Am Soc Nephrol 2015; 10: 703-709.

[7] Ma J, Pan X, Wang Z, Wang Y, Feng X, Ren H, Zhang W, Chen X, Wang W and Chen N. Twenty-one novel mutations identified in the COL4A5 gene in Chinese patients with X-linked Alport's syndrome confirmed by skin biopsy. Nephrol Dial Transplant 2011; 26: 4003-4010.

[8] Murata T, Katayama K, Oohashi T, Jahnukainen T, Yonezawa T, Sado Y, Ishikawa E, Nomura S, Tryggvason K and Ito M. COL4A6 is dispensable for autosomal recessive Alport syndrome. Sci Rep 2016; 6: 29450.

[9] Xiang R, Fan LL, Huang H, Cao BB, Li XP, Peng DQ and Xia K. A novel mutation of GATA4 (K319E) is responsible for familial atrial septal defect and pulmonary valve stenosis. Gene 2014; 534: 320-323.

[10] Xiang R, Fan LL, Huang H, Zhao SP and Chen YQ. Whole-exome sequencing identifies a nov-

el mutation of DSG2 (Y198C) in a Chinese arrhythmogenic right ventricular cardiomyopathy patient. Int J Cardiol 2016; 214: 1-3.

[11] Nozu K, Iijima K, Ohtsuka Y, Fu XJ, Kaito H, Nakanishi K and Vorechovsky I. Alport syndrome caused by a COL4A5 deletion and exonization of an adjacent AluY. Mol Genet Genomic Med 2014; 2: 451-453.

[12] Guo Y, Yuan J, Liang H, Xiao J, Xu H, Yuan L, Gao K, Wu B, Tang Y, Li X and Deng H. Identification of a novel COL4A5 mutation in a Chinese family with X-linked Alport syndrome using exome sequencing. Mol Biol Rep 2014; 41: 3631-3635.

[13] Zhou J, Hertz JM, Leinonen A and Tryggvason K. Complete amino acid sequence of the human alpha 5 (IV) collagen chain and identification of a single-base mutation in exon 23 converting glycine 521 in the collagenous domain to cysteine in an Alport syndrome patient. J Biol Chem 1992; 267: 12475-12481.

[14] Harvey SJ, Zheng K, Sado Y, Naito I, Ninomiya Y, Jacobs RM, Hudson BG and Thorner PS. Role of distinct type IV collagen networks in glomerular development and function. Kidney Int 1998; 54: 1857-1866.

[15] Wang Q, Liu F, Xing Y, Wei X, Li H, Zhang S, Liu J, Wang Q, Tang Z and Liu M. Mutation c.359_363delGTATTinsATAC in the COL4A5 causes Alport syndrome in a Chinese family. Gene 2013; 512: 482-485.

[16] Savige J, Colville D, Rheault M, Gear S, Lennon R, Lagas S, Finlay M and Flinter F. Alport syndrome in women and girls. Clin J Am Soc Nephrol 2016; 11: 1713-20.

[17] Heidet L and Gubler MC. The renal lesions of Alport syndrome. J Am Soc Nephrol 2009; 20: 1210-1215.

[18] Xiu X, Yuan J, Deng X, Xiao J, Xu H, Zeng Z, Guan L, Xu F and Deng S. A novel COL4A5 mutation identified in a Chinese Han family using exome sequencing. Biomed Res Int 2014; 2014: 186048.

[19] Artuso R, Fallerini C, Dosa L, Scionti F, Clementi M, Garosi G, Massella L, Epistolato MC, Mancini R, Mari F, Longo I, Ariani F, Renieri A and Bruttini M. Advances in Alport syndrome diagnosis using next-generation sequencing. Eur J Hum Genet 2012; 20: 50-57.