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CLINICAL UTILITY GENE CARD UPDATE

Clinical utility gene card for: Alport syndrome – update 2014

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1. DISEASE CHARACTERISTICS

1.1 Name of the Disease (Synonyms)

Alport syndrome.

1.2 OMIM# of the Disease

301050 (X-linked form), 203780 (autosomal recessive form), 104200 (autosomal dominant form).

1.3 Name of the Analysed Genes or DNA/Chromosome Segments

COL4A5, COL4A4, COL4A3.

1.4 OMIM# of the Gene(s)

303630 (*COL4A5*), 120131 (*COL4A4*), 120070 (*COL4A3*).

1.5 Mutational Spectrum

COL4A5: Missense variants (~45% of the variants, 85% of which are glycine substitutions in the conserved Gly-Xaa-Yaa repeat sequence in the collagenous domain of the alpha5(IV)-chain); splice site variants (~20%); frame shifts (small deletions, small insertions/duplications) (~20%); nonsense variants (~7%); large deletions and insertions/duplications (~12%) and other larger structural rearrangements (inversions and translocations). More than 1168 unique variants are known.

COL4A4: Missense variants (~50% of the variants, 75% of which are glycine substitutions in the conserved Gly-Xaa-Yaa repeat sequence in the collagenous domain of the alpha4(IV)-chain); frame shifts (small deletions, small insertions/duplications) (~27%); splice site variants (~13%); nonsense variants (~9%) and larger deletions. More than 268 unique variants are known.

COL4A3: Missense variants (~45% of the variants, 85% of which are glycine substitutions in the conserved Gly-Xaa-Yaa repeat sequence in the collagenous domain of the alpha3(IV)-chain); frame shifts (small deletions, small insertions/duplications) (~20%); nonsense variants (~15%); splice site variants (~15%) and larger deletions. More than 266 unique variants are known.

See:

HGMD (http://www.hgmd.org)

Alport LOVD databases (*COL4A5*, *COL4A4*, *COL4A3*) (https://grenada.lumc.nl/LOVD2/COL4A/home.php?action=switch_db)

ARUP Alport (*COL4A5*) database (www.arup.utah.edu/database/ALPORT/ALPORT_welcome.php)

1.6 Analytical Methods

Targeted next generation sequencing. Direct sequencing of genomic exonic DNA, including flanking intronic sequences. Reverse transcriptase PCR analysis of mRNA extracted from cultured skin fibroblasts or hair roots for deep intronic variants causing RNA splicing aberrations (X-linked form). Multiplex ligation-dependent probe amplification (MLPA) for the detection of submicroscopic deletions and duplications. Conventional cytogenetic analysis for the detection of structural chromosome rearrangements.

1.7 Analytical Validation

Confirmation in an independent biological sample from the index case or an affected relative. Single exon deletions and duplications detected by MLPA should be confirmed with another technique.

1.8 Estimated Frequency of the Disease

(Incidence at birth ('birth prevalence') or population prevalence)

The most widely used estimate of the prevalence of Alport syndrome is 1:5 000 based on the finding of about 300 cases in Utah and southern Idaho in a population of 1 500 000 people.[1] The incidence of Alport syndrome was found to be 1:53 000 in Finland[2] and 1:17 000 in southern Sweden.[3] The inheritance is X-linked in ~85% of the families, autosomal recessive in ~15% and autosomal dominant in very few families.

1.9 If applicable, prevalence in the ethnic group of investigated person

Not applicable.

1.10 Diagnostic Setting

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive testing	☒	☐
C. Risk assessment in relatives	☒	☐
D. Prenatal	☒	☐

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Comment: Genetic analysis is the diagnostic method of choice because it enables screening of symptomatic or at-risk family members, and may obviate the need for a kidney biopsy or in case electron microscopy of glomeruli is not available.

Genetic analysis is mainly used for confirmation of a clinical diagnosis and in relation to genetic counselling.

Prenatal diagnosis and pre-implantation genetic diagnosis are available for families with a known sequence variant.

2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives	C: False negatives
			B: False positives	D: True negatives
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A+C)
			Specificity:	D/(D+B)
Negative	C	D	Positive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

2.1 Analytical Sensitivity
(proportion of positive tests if the genotype is present)

COL4A5: Sanger sequencing of genomic DNA and MLPA: Above 99%.

COL4A3 and *COL4A4*: Sanger sequencing of genomic DNA and MLPA: Probably above 99%.

Targeted next generation sequencing of all the three genes (COL4A5, COL4A4 and COL4A3): Probably above 99%.

2.2 Analytical Specificity
(proportion of negative tests if the genotype is not present)

Sanger sequencing and MLPA of *COL4A5*: Above 99%.

Sanger sequencing and MLPA of *COL4A3* and *COL4A4*: Probably above 99%.

Targeted next generation sequencing of all the three genes (COL4A5, COL4A4 and COL4A3): Probably above 99%.

2.3 Clinical Sensitivity
(proportion of positive tests if the disease is present)

The clinical sensitivity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

X-linked form: Highly dependent on fulfilment of the clinical criteria for Alport syndrome[4] and may be supported by immunohistochemical findings in a kidney or skin biopsy. Above 80% in families fulfilling three or more diagnostic criteria. Higher in families with obvious X-linked inheritance, and higher in males than in females.[5]

Autosomal recessive form: Highly dependent on fulfilment of the clinical criteria for Alport syndrome[4] and may be supported by immunohistochemical findings in a kidney or skin biopsy. Above 80% in families fulfilling three or more diagnostic criteria. Higher in families with consanguinity.

2.4 Clinical Specificity
(proportion of negative tests if the disease is not present) The clinical specificity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

X-linked form: Almost 100% in males by the age of 20 years.

Autosomal recessive form: Approaches 100% in males and females by the age of 20 years.

2.5 Positive clinical predictive value
(life time risk to develop the disease if the test is positive).

X-linked form: Almost 100% with respect to end-stage renal disease (ESRD) in males, and 30–40% in females. The risk for high-tone sensorineural hearing loss is 80–90% in males and 30% in females. The risk for ophthalmological manifestations is 30–50% for retinopathy and 15–30% for lenticonus in males, and lower in females.

Autosomal recessive form: Almost 100%. Heterozygotes may develop thin basement membrane disease/familial haematuria ± proteinuria and sometimes renal impairment (OMIM #141200). The frequency of high-tone sensorineural hearing loss and ophthalmological manifestations are about the same as seen for males with X-linked form.

2.6 Negative clinical predictive value
(Probability of not developing the disease if the test is negative). Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in that family had been tested:

X-linked: Almost 100%.

Autosomal recessive: Almost 100%. Heterozygotes may develop thin basement membrane disease/benign familial haematuria (OMIM #141200)

Index case in that family had not been tested:

X-linked: Unknown, but probably high.

Autosomal recessive: Unknown, but probably high.

3. CLINICAL UTILITY

3.1 (Differential) diagnostics: The tested person is clinically affected
(To be answered if in 1.10 'A' was marked)

3.1.1 Can a diagnosis be made other than through a genetic test?

No	☐ (continue with 3.1.4)	
Yes	☒	
	Clinically	☒
	Imaging	☐
	Endoscopy	☐
	Biochemistry	☐
	Electrophysiology	☐
	Other (please describe)	☒ Family history and a clinical examination including urine analysis, renal function study, ophthalmoscopy and audiometry. Kidney biopsy: Electron microscopy for characteristic ultrastructural changes, and immunohistochemical staining for the alpha3-, alpha4- and alpha5-chains of type IV collagen. All the three chains are absent in the glomerular basement membrane (GBM), Bowman's capsules and tubular basement membranes in 70–80% of males with the X-linked form. In the autosomal forms of Alport syndrome, all the three chains are also absent from the GBM in most patients, but the alpha5-chain is present in Bowman's capsules and tubular basement membranes. Skin biopsy: Immunohistochemical staining for the alpha5(IV)-chain. Absent in the epidermal basement membrane in 70–80% of males with the X-linked form. Recommended diagnostic procedure: 1. Family history and clinical examination

2. Audiogram and eye examination by slit lamp ophthalmoscopy
3. Targeted next generation sequencing of *COL4A3*, *COL4A4*, *COL4A5* for sequence variants including large deletions/duplications.[6,7] Alternatively:
4a. X-linked form: Skin biopsy for immunohistochemical staining for the alpha5(IV)-chain and RT-PCR analysis of mRNA from cultured fibroblasts, or, alternatively, direct sequencing of all 53 *COL4A5* exons on genomic DNA. The immunohistochemical staining may be associated with both false negative and false positive results
4b. MLPA analysis of genomic DNA for *COL4A5* deletion/duplication.[8]
4c. Autosomal forms: Direct sequencing of *COL4A4* and *COL4A3* on genomic DNA in case of normal immunohistochemical staining for the alpha5(IV)-chain of the skin biopsy and no *COL4A5* variant or if the family history suggests autosomal inheritance (eg, consanguineous parents)
4d. MLPA analysis of genomic DNA for *COL4A3* deletion/duplication
5. Consider a kidney biopsy.

3.1.2 Describe the burden of alternative diagnostic methods to the patient

Kidney biopsy confers a risk to the patient (bleeding, infection and pain) and a skin biopsy leaves a tiny scar.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?

Genetic testing is useful for confirming a clinical diagnosis, informing genetic counselling and facilitating prenatal diagnosis and pre-implantation genetic diagnosis. The three type IV collagen genes, COL4A5, *COL4A3* and *COL4A4*, are very large genes with 48–53 exons and a coding sequence of more than 5000 bp. Targeted next generation sequencing will lower the costs and turn-around time, and will identify some large *COL4A3* and *COL4A4* rearrangements that are not detected by Sanger sequencing.

3.1.4 Will disease management be influenced by the result of a genetic test?

No	☐	
Yes	☒	
	Therapy (please describe)	It is recommended that patients with Alport syndrome should be treated with renin–angiotensin system blockade if they develop hypertension and/or proteinuria, and it is possible that such treatment may be indicated even earlier than this.[9]
	Prognosis (please describe)	Nonsense variants, frame shifts and larger structural rearrangements are associated with a younger age at ESRD as compared with missense variants. The first group of variants confer on an affected male patient a probability of 90% of developing ESRD before the age of 30 years. The risk is 50% for patients with a missense variant.[10] Contiguous deletions across a variable portion of the 5′-end of *COL4A5* and the first two exons of the neighbouring *COL4A6* gene are associated with Alport syndrome with diffuse leiomyomatosis (OMIM #308940). The AMME complex (Alport syndrome, mental retardation, midtface hypoplasia and elliptocytosis; OMIM #300194) is a contigious gene deletion syndrome, which includes deletion of *COL4A5* together with neighbouring genes.
	Management (please describe)	Interdiciplinary clinical follow-up at renal/paediatric, audiological and ophthalmological clinics. Genetic counselling.

3.2 Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history (To be answered if in 1.10 'B' was marked)

3.2.1 Will the result of a genetic test influence lifestyle and prevention?

If the test result is positive (please describe). If the test is positive, clinical follow-up and genetic counselling should be recommended. Careful management of blood pressure in a patient with a positive predictive test significantly prolongs the functioning life of a patient's native kidneys and postpones the need for renal replacement therapy. In addition, children should be referred for audiological examination to assess the need for a hearing aid.

Some patients may elect to have prenatal diagnosis or pre-implantation genetic diagnosis if they wish to avoid having an affected child.

If the test result is negative (please describe). This is not relevant if the test is negative for a known familial variant.

3.2.2 Which options in view of lifestyle and prevention does a person at risk have if no genetic test has been done (please describe)?

The same interdisciplinary surveillance programme comprising nephrological/paediatric, ophthalmological and audiological examination as for those with a positive genetic test. The frequency depends on the clinical findings.

3.3 Genetic risk assessment in family members of a diseased person

(To be answered if in 1.10 'C' was marked)

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?

Yes. The risk assessment depends on the mode of inheritance; X-linked, autosomal recessive or dominant.

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?

Yes. If a pathogenic variant has been identified in the index patient, genetic counselling and testing for the familial variant could be offered to relatives at risk. If a relative tests negative, then none of their offspring are at risk and they will not need to be tested.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?

Yes. Carrier testing and presymptomatic testing in relatives will be possible.

3.4 Prenatal diagnosis

(To be answered if in 1.10 'D' was marked)

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?

Yes, prenatal and pre-implantation diagnostics can be performed in the family, if requested.

4. IF APPLICABLE, FURTHER CONSEQUENCES OF TESTING

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe)

Genetic testing can be useful for the patient/family in relation to genetic counselling. Genetic testing of relatives can also be relevant in relation to the selection of potential family donors for kidney transplantation. The affected mothers of males with X-linked Alport syndrome are usually advised not to donate a kidney because of their own risk of kidney failure.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Hasstedt SJ, Atkin CL: X-linked inheritance of Alport syndrome: Family P revisited. *Am J Hum Genet* 1983; **35**: 1241–1251.

2 Pajari H, Kaariainen H, Muhonen T, Koskimies O: Alport's syndrome in 78 patients: epidemiological and clinical study. *Acta Paediatr* 1996; **85**: 1300–1306.

3 Persson U, Hertz JM, Wieslander J, Segelmark M: Alport syndrome in southern Sweden. *Clin Nephrol* 2005; **64**: 85–90.

4 Flinter FA, Cameron JS, Chantler C, Houston I, Bobrow M: Genetics of classic Alport's syndrome. *Lancet* 1988; **2**: 1005–1007.

5 Hanson H, Storey H, Pagan J, Flinter F: The value of clinical criteria in identifying patients with X-linked Alport syndrome. *Clin J Am Soc Nephrol* 2011; **6**: 198–203.

6 Artuso R, Fallerini C, Dosa L *et al*: Advances in Alport syndrome diagnosis using next-generation sequencing. *Eur J Hum Genet* 2012; **20**: 50–57.

7 Morinière V, Dahan K, Hilbert P *et al*: Improving Mutation Screening in Familial Hematuric Nephropathies through Next Generation Sequencing. *J Am Soc Nephrol* 2014; e-pub ahead of print 22 May 2014.

8 Hertz JM, Juncker I, Marcussen N: MLPA and cDNA analysis improves COL4A4 mutation detection in X-linked Alport syndrome. *Clin Genet* 2008; **74**: 522–530.

9 Savige J, Gregory M, Gross O, Kashtan C, Ding J, Flinter F: Expert guidelines for the management of Alport syndrome and thin basement membrane nephropathy. *J Am Soc Nephrol* 2013; **24**: 364–375.

10 Jais JP, Knebelmann B, Giatras I *et al*: X-linked Alport syndrome: natural history in 195 families and genotype- phenotype correlations in males. *J Am Soc Nephrol* 2000; **11**: 649–657.