the children's hospital at Westmead

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Department of Molecular Genetics

Australian Renal Gene Panels by Massively Parallel Sequencing

Genetic and inheritable renal disorders are a large and heterogenous group of disorders both genetically and phenotypically. The Australian Renal Gene Panels contain genes known to cause many of these disorders.

Important notice for MPS testing: We require a specific **MPS request form** for each sample, in order to assist with interpretation of results. Please contact the laboratory at molgenlab.schn@health.nsw.gov.au for a copy.

While MPS analysis will be restricted to the genes listed in the panel there remains a small risk of incidental findings and should be discussed with the patient prior to testing. It is also important to note that mutations in some of these genes are phenotypically heterogeneous and may be associated with non-renal disorders. These variants will be reported. It is important in the consent process for the patient to be aware of this. **Written consent for genetic testing** from the patient must be obtained prior to testing and a copy **must be forwarded to the laboratory**. The original should be retained with the patient's medical record. The CHW Genetic Testing Informed Consent Form is available if a local consent form is unavailable; please contact the laboratory for a copy. Testing will NOT commence unless this form has been received.

Pricing and Turnaround times

Index case (Proband)

Renal gene panel testing using Massively Parallel Sequencing (MPS) Genes in bold will be gap filled by Sanger sequencing if <20x coverage		
Panel	**Price AUD$**	**Turnaround time**
Atypical Hemolytic uremic syndrome (aHUS) panel ***CFH, CD46, CFI, CFB***, *C3, THBD, MMACHC*	$1,500	16 weeks
Alport syndrome panel ***COL4A3, COL4A4, COL4A5***	$1,500	16 weeks
Nephrotic syndrome panel ***NPHS1, NPHS2, WT1***, *PLCE1, LAMB2, PTPRO, ACTN4, TRPC6, CD2AP, APOL1, INF2, MYO1E, PAX2, ALMS1, ARHGAP24, COQ2, COQ6, ITGA3, ITGB4, LMX1B, MYH9, PDSS2, SCARB2, COL4A3, COL4A4, COL4A5, SMARCAL1, LMNA, TTC21B*	$1,800	16 weeks
Hyperoxaluria panel ***AGXT, GRHPR, HOGA1***	$1,200	16 weeks
Cystinosis panel *CTNS – see below* for Sanger sequencing. A 57kb deletion has been reported in up to 76% of affected Northern European alleles. Other large deletions have been reported. It is recommended that a suitable CGH array be performed to detect large deletions prior to Sanger sequencing		

Service includes DNA extraction (if required), TruSight target enrichment and library preparation, sequencing on Illumina HiSeq, bioinformatics analysis and variant interpretation. Sanger confirmation will be carried out for likely pathogenic mutation(s), such as those previously reported in the literature, mutations in domains associated with a disorder/phenotype with a coding change predicted to be damaging (based on in silico predictions), nonsense or frameshifting mutations, and changes affecting canonical splice sites. Other variants of uncertain significance (VOUS) will only be confirmed by Sanger sequencing upon request (for an additional charge).

Additional services		
Description	**Price AUD$**	**Turnaround time**
Additional Sanger confirmation for VOUS	$300 per variant	4 weeks
Re-analysis of sequencing data for additional genes/panel	Price on inquiry	4 weeks
Additional Sanger sequencing for "gap-filling"	$100 per gap	4 weeks
Sanger sequencing of *CTNS*	$600	6 weeks
Sanger sequencing of *DGKE* (aHUS)	$500	6 weeks

Cascade testing

Once a family-specific mutation has been identified, cascade testing can be used for at-risk family members.

Cascade testing (known mutation)		
Sample description	**Price AUD$**	**Turnaround time**
Single patient (Sanger sequencing)	$250	4 weeks
Multiple patients (Sanger sequencing) More than one patient tested at the same time for the same familial mutation	$200	4 weeks
Predictive testing 2nd sample (Sanger sequencing) 2nd sample received after 1st sample has been tested	$100	4 weeks
Prenatal testing of familial mutation (Sanger sequencing)	$600	<2 weeks

Methodology

Illumina TruSight technology is used to perform gene targeting and library enrichment. The enrichment kit currently employed is the TruSight One panel (FC-141-1006). The full lists of genes can be found on the Illumina website (www.illumina.com/clinical/translational_genomics/panels.ilmn). Target regions of interest are restricted to coding regions and the canonical splice sites. Library sequencing will be performed using Illumina HiSeq 2500. The average performance for a sample meeting the appropriate quality control parameters (see Specimen) is an average coverage of 200x reads over the entire TruSight panel, equivalent to a yield of >97% of target bases with at least 20x coverage. Performance may vary across different gene sets.

Raw data will be aligned and variants will be called using SoftGenetics NextGene. The variants investigated will be limited to the genes/panel requested to minimize the risk of incidental findings. Raw data will be stored for 5 years and re-analysis can be carried out for additional genes when requested. Variants identified will be annotated using AlamutHT and potential for pathogenicity will be assessed using Alamut interpretation software. Only mutations deemed likely to be pathogenic will be confirmed by Sanger sequencing. Additional Sanger confirmation of variants will incur extra charges.

A report will be generated to provide information on any putative pathogenic variants and to list any variants of

uncertain significance (defined as having an allele frequency of <0.1% for dominant disorders, or <1% for recessive disorders; with a predicted coding consequence). The report will state parameters of sequencing performance, such as the average depth of coverage and the target rate for each of the genes of interest.

Limitations

Substitutions and most small insertions and deletions will be detected. However some genetic abnormalities such as copy number variations (CNV) including large deletions and duplications, inversions and rearrangements cannot be detected. Apart from canonical splice sites, pathogenic intronic variants may not be covered or identified. Some genes may have high homology to other regions in the genome and this may mask or prevent variants being identified.

# indicates the gaps (bases where coverage is <20x) in the gene will be filled by Sanger sequencing where clear pathogenic variant/s that can account for the patient's phenotype have NOT been detected.

* Expected target rate indicates the percentage of target bases within a particular gene that will be sequenced at a minimum of 20x coverage. Figures provided for illustration purposes only and actual performance may vary between samples.

aHUS panel				
Gene	Associated phenotype	OMIM number	Mode of inheritance	Expected target rate (%)*
***CFH**#*	{Hemolytic uremic syndrome, atypical, susceptibility to, 1}	235400	AD	100%
	Complement factor H deficiency	609814	AR	
	Basal laminar drusen	126700	AR	
	{Macular degeneration, age-related, 4}	610698	?AD	
***CD46**#*	{Hemolytic uremic syndrome, atypical, susceptibility to, 2}	612922	AD	99.0%
***CFI**#*	{Hemolytic uremic syndrome, atypical, susceptibility to, 3}	612923	AD	96.3%
	Complement factor I deficiency	610984	AR	
	{Macular degeneration, age-related, 13, susceptibility to}	615439	AD	
***CFB**#*	{Hemolytic uremic syndrome, atypical, susceptibility to, 4}	612924	AD	96.7%
	Complement factor B deficiency	615561	AR	
C3	{Hemolytic uremic syndrome, atypical, susceptibility to, 5}	612925	AD	99.8%
	C3 deficiency	613779	AR	
	{Macular degeneration, age-related, 9}	611378	AD	
THBD	{Hemolytic uremic syndrome, atypical, susceptibility to, 6}	612926	AD	98.0%
	Thrombophilia due to thrombomodulin defect	614486	AD	
MMACHC	Methylmalonic aciduria and homocystinuria, cblC type	277400	AR	81.7%
On request (addition costs apply – see above)				
C5	[Eculizumab, poor response to] (variant at p.Arg885)	615749	AD	Specific variant
DGKE	Nephrotic syndrome, type 7	615008	AR	By Sanger sequencing
	aHUS/C3 Glomerulopathy		AR	

Alport panel				
Gene	**Associated phenotype**	**OMIM number**	**Mode of inheritance**	**Expected target rate (%)***
COL4A#3	Alport syndrome, autosomal dominant	104200	AD	100%
	Alport syndrome, autosomal recessive	203780	AR	
	Thin basement membrane disease (Hematuria, benign familial)	141200	AD	
COL4A4#	Alport syndrome, autosomal recessive	203780	AR	96.6%
	Thin basement membrane disease (Hematuria, benign familial)	141200	AD	
COL4A5#	Alport syndrome	301050	X-linked	100%
NPHS2	On request - R229Q variant			

Nephrotic syndrome panel				
Gene	**Associated phenotype**	**OMIM number**	**Mode of inheritance**	**Expected target rate (%)***
NPHS1#	Nephrotic syndrome, type 1	256300	AR	99.1%
NPHS2#	Nephrotic syndrome, type 2	600995	AR	96.6%
WT1 #	Nephrotic syndrome, type 4	256370	AD	97.9%
	Denys-Drash syndrome	194080	AD	
	Frasier syndrome	136680	AD	
	Meacham syndrome	608978	AD	
	Mesothelioma, somatic	156240	AD	
	Wilms tumor, type 1	194070	AD	
PLCE1	Nephrotic syndrome, type 3	610725	AR	93.8%
LAMB2	Nephrotic syndrome, type 5, with or without ocular abnormalities	614199	AR	93.8%
PTPRO	Nephrotic syndrome, type 6	614196	AR	99.4%
ACTN4	Glomerulosclerosis, focal segmental, 1	603278	AD	98.4%
TRPC6	Glomerulosclerosis, focal segmental, 2	603965	AD	93.2%
CD2AP	Glomerulosclerosis, focal segmental, 3	607832	AD	100%
APOL1	{Glomerulosclerosis, focal segmental, 4, susceptibility to}	612551	AD	100%
INF2	Glomerulosclerosis, focal segmental, 5	613237	AD	87.3%
MYO1E	Glomerulosclerosis, focal segmental, 6	614131	AR	98.1%
PAX2	Glomerulosclerosis, focal segmental, 7	616002	AD	100%
ALMS1	Alstrom syndrome	203800	AR	96.8%
ARHGAP24	Familial focal segmental glomerulosclerosis.		AD	90.6%
COQ2	Coenzyme Q10 deficiency, primary, 1	607426	AR	99.9%
COQ6	Coenzyme Q10 deficiency, primary, 6	614650	AR	100%
ITGA3	Interstitial lung disease, nephrotic syndrome, and epidermolysis	614748	AR	100%
ITGB4	Epidermolysis bullosa of hands and feet	131800	AD	94.0%
	Epidermolysis bullosa, junctional, non-Herlitz type	226650	AR	
	Epidermolysis bullosa, junctional, with pyloric atresia	226730	AR	
LMX1B	Nail-patella syndrome	161200	AD	98.6%
MYH9	May-Hegglin anomaly	155100	AD	97.1%
	Epstein syndrome	153650	AD	
	Fechtner syndrome	153640	AD	

	Macrothrombocytopenia and progressive sensorineural deafness	600208	AD	
	Sebastian syndrome	605249	AD	
	Deafness, autosomal dominant 17	603622	AD	
PDSS2	Coenzyme Q10 deficiency, primary, 3	614652	AR	97.1%
SCARB2	Epilepsy, progressive myoclonic 4, with or without renal failure	254900	AR	100%
COL4A3	Alport syndrome, autosomal dominant	104200	AD	100%
	Alport syndrome, autosomal recessive	203780	AR	
	Thin basement membrane disease (Hematuria, benign familial)	141200	AD	
COL4A4	Alport syndrome, autosomal recessive	203780	AR	96.6%
	Thin basement membrane disease (Hematuria, benign familial)	141200	AD	
COL4A5	Alport syndrome	301050	X-linked	100%
SMARCAL1	Schimke immunoosseous dysplasia	242900	AR	98.2%
LMNA	Cardiomyopathy, dilated, 1A	115200	AD	94.0%
	Charcot-Marie-Tooth disease, type 2B1	605588	AR	
	Emery-Dreifuss muscular dystrophy 2, AD	181350	AD	
	Emery-Dreifuss muscular dystrophy 3, AR	181350	AR	
	Heart-hand syndrome, Slovenian type	610140	AD	
	Hutchinson-Gilford progeria	176670	AD	
	Lipodystrophy, familial partial, 2	151660	AD	
	Malouf syndrome	212112	AD	
	Mandibuloacral dysplasia	248370	AR	
	Muscular dystrophy, congenital	613205	AD	
	Muscular dystrophy, limb-girdle, type 1B	159001	AD	
	Restrictive dermopathy, lethal	275210	AD	
TTC21B	Nephronophthisis 12	613820	AR	100%
	Short-rib thoracic dysplasia 4 with or without polydactyly	613819	AR	

Hyperoxaluria panel

Gene	Associated phenotype	OMIM number	Mode of inheritance	Expected target rate (%)*
AGXT	Hyperoxaluria, primary, type 1	259900	AR	100%
GRHPR	Hyperoxaluria, primary, type II	260000	AR	100%
HOGA1	Hyperoxaluria, primary, type III	613616	AR	100%

Cystinosis

Gene	Associated phenotype	OMIM number	Mode of inheritance	Expected target rate (%)*
CTNS	Cystinosis, atypical nephropathic	219800	AR	Sanger sequencing
	Cystinosis, late-onset juvenile or adolescent nephropathic	219900	AR	
	Cystinosis, nephropathic	219800	AR	
	Cystinosis, ocular nonnephropathic	219750	AR	

Specimen

2 to 5mls EDTA whole blood or extracted DNA from blood with at least 2 unique identifiers (Minimum amount of DNA 2 µg, minimum concentration 100 ng/µL, ratio of A260 to A280 greater than 1.8). Stored DNA may be used, but we may request a new sample if the quality is below the parameters listed. DNA from some sources (FFPE, blood spots) or DNA with suboptimal quality may result in test performance below the statistics quoted. In these cases, gap-filling by Sanger sequencing will not be provided. Please contact the laboratory for further information.

Billing

Please indicate who should be invoiced for the testing. The organisation or the individual must have agreed to pay for the testing. Testing will not commence without billing consent.

Clinical and Counselling Services

Within Australia details of the clinical and counselling services available in your area can be obtained from your State Health service or via links from the NSW Genetic Education Program ph [02] 9926 7324; fax [02] 9906 7529; www.genetics.com.au

Transport

Please transport the specimens at room temperature. Referring laboratories will be responsible for arranging and paying for the transportation of specimens to the laboratory. For countries outside of Australia, blood and DNA samples without a known infectious risk, do not need quarantine inspection if external packaging is clearly labeled, i.e. "Contents: Product of human origin, non-hazardous, non-infectious, for diagnostic in-vitro testing only" and "Exempt human specimen. Diagnostic specimens packed in compliance with IATA packaging instructions 650". If package is not correctly labeled and incurs a quarantine charge, this fee will be passed onto the referring laboratory. See http://www.daff.gov.au/biosecurity/import

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