Preconception genetic carrier screening in an Australian fertility clinic, the first 1000 patients

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Virtus Diagnostic Genetics provides preconception genetic carrier screening for patients using a NATA accredited Illumina Inherited Disease screening panel[1]. This panel contains 552 genes for a total of 592 rare diseases. ACMG guidelines are utilised to interpret and report pathogenic/likely pathogenic variants. For this study, 1095 patients were screened/reported.

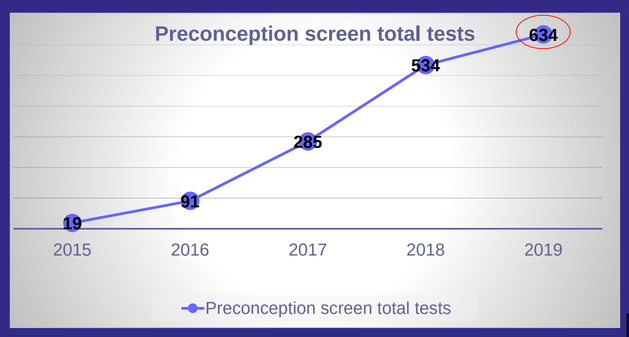

Figure 1. Total screen requests, 2019 projected total circled in red.

	Total	Females	Males	Gamete Donors
Total Patients Screened	1095	612	483	180
Patients with variants	676	380	296	107
Patients without variants	419	232	187	73
% with variant	61.7	62.1	61.3	59.4

Table 1. Total patients screened, reports with and without pathogenic/likely pathogenic variants

In the 1095 patients (table 1), 676 pathogenic/likely pathogenic variants in 252 genes were reported. 56% were female patients and 44% were male patients. 61% of all patients had at least a single variant to report. 101 genes had a single variant reported. 419 patients including 73 donors had no variant.

These 1095 patients present a reasonable overview of the variants present within non affected individuals in the Australian population. Interpreting and reporting any variant without a disease phenotype is a challenge.

Gene	Number of times reported (of 1095)	Reported in Females	Reported in Males	Number of Different Variants reported	Carrier rate in Virtus screen	Estimated Incidence of affected individuals	Calculated carrier rate	Disease
CFTR	72	47	25	35	1 in 15	1 in 2500	1 in 26	Cystic Fibrosis
GJB2	58	36	22	13	1 in 19	1 in 500	1 in 12	Nonsyndromic hearing loss
PAH	34	21	13	15	1 in 32	1 in 10,000	1 in 51	Phenylketonuria
CBS	31	18	13	6	1 in 35	1 in 200,000	1 in 224	Homocystinuria
ATP7B	23	10	13	17	1 in 48	1 in 30,000	1 in 87	Wilson disease
POLG	22	13	9	11	1 in 50	1 in 40,000	1 in 100	Leigh syndrome
DPYD	20	9	11	5	1 in 55	rare/unknown	Drug interaction	Dihydropyrimidine dehydrogenase deficiency, toxic reactions to fluoropyrimidine (2 to 8% of population)
PMM2	21	10	11	3	1 in 52	1 in 20,000	1 in 71	PMM2-congenital disorder of glycosylation
PKLR	18	9	9	3	1 in 61	1 in 20,000	1 in 71	Pyruvate kinase deficiency
PKHD1	17	4	13	12	1 in 64	1 in 20,000	1 in 71	Polycystic kidney disease
SLC22A5	17	10	7	6	1 in 64	1 in 100,000	1 in 159	Primary carnitine deficiency
MEFV	16	12	4	8	1 in 68	1 in 10,000	1 in 51	Familial Mediterranean fever
SMN1* (*MLPA)	16	11	5	2	1 in 68	1 in 8000	1 in 45	Spinal muscular atrophy
ABCA12	15	4	11	2	1 in 73	1 in 1,000,000	1 in 500	Autosomal recessive congenital ichthyosis
SLC37A4	15	10	5	4	1 in 73	1 in 100,000	1 in 159	Glycogen storage disease type I
GBA	15	9	6	6	1 in 73	1 in 50,000	1 in 112	Gaucher disease
CDH23	14	9	5	6	1 in 78	1 in 100,000	1 in 159	Usher syndrome type 1
USH2A	13	7	6	10	1 in 84	1 in 100,000	1 in 159	Usher syndrome type 2
ALDOB	11	5	6	3	1 in 100	1 in 20,000	1 in 71	Hereditary fructose intolerance
GNRHR	11	7	4	7	1 in 100	rare/unknown	multiple genes	Hypogonadotropic hypogonadism 7 with or without anosmia

Table 2. The top 20 most reported genes in the 1095 patient reports. Female, male and number of different variants within each gene is shown. Calculated carrier rate from within the 1095 patients compared to the estimated incidence (sources include ClinGen, ClinVar, OMIM, Orphanet, NCBI) and the calculated carrier rate from the incidence frequency.

The Australian population is a diverse genetic population and the majority of patients screened in this test had no significant phenotype, other than fertility issues. The screening was conducted on a private fee for service basis (figure 1), with continued growth and uptake of testing. Reports were returned to the requesting clinician. Table 2 presents the top 20 genes reported. Each gene in this table had a carrier rate of at least 1 in 100. CFTR was reported the most often, with 35 different variants reported and a carrier rate of 1 in 15. Both non-syndromic hearing loss (GJB2) and phenylketonuria (PAH) are also common with 1 in 19 and 1 in 32 carrier rates respectively. This mirrors closely the expectation as calculated by live birth affected individuals. Autosomal recessive congenital ichthyosis (ABCA12) may be over represented in our data, either due to an increased risk of miscarriage or incorrectly classified variants reported in the gene. The presence of hypogonadotropic hypogonadism (GNRHR) in the top 20 gene list may provide an avenue for further investigation within the infertile population.

The genetic screening of gamete donors is now an expectation within a donor IVF cycle. The larger screening panel enables a wide set of genes to be analysed and carrier status ascertained. The corollary of a larger panel are more donor carriers are identified. Genetic counselling is a key component of the genetic testing. 11 same sex female partners were screened and 1 same sex male couple screened with a female gamete donor, who was also acting as the surrogate mother for the pregnancy.

Couple	Gene	Variant	Disorder
1	CBS	NM_001178008.1:c.833T>C p.(Ile278Thr)	Homocystinuria
	CBS	NM_001178008.1:c.833T>C p.(Ile278Thr)	Homocystinuria
2	CFTR	NM_000492.3:c.1521_1523delCTT p.(Phe508del)	Cystic Fibrosis
	CFTR	NM_000492.3:c.1521_1523delCTT p.(Phe508del)	Cystic Fibrosis
3	CFTR	NM_000492.3:c.650A>G p.(Glu217Gly)	CBAVD and Pancreatitis, Asian ancestry
	CFTR	NM_000492.3:c.650A>G p.(Glu217Gly)	CBAVD and Pancreatitis, Asian ancestry
4	CFTR	NM_000492.3:c.1521_1523delCTT p.(Phe508del)	Cystic Fibrosis
	CFTR	NM_000492.3:c.3528delC p.(Lys1177SerfsTer15)	Cystic Fibrosis
5	CFTR	NM_000492.3:c.1521_1523delCTT p.Phe508del	Cystic Fibrosis
	CFTR	NM_000492.3:c.3154T>G p.(Phe1052Val)	Cystic Fibrosis
6	CFTR	NM_000492.3:c.1521_1523delCTT p.(Phe508del)	Cystic Fibrosis
	CFTR	NM_000492.3:c.1521_1523delCTT p.(Phe508del)	Cystic Fibrosis
7	DHCR7	NM_001360.2:c.1A>G p.(Met1Val)	Smith-Lemli-Optiz syndrome
	DHCR7	NM_001360.2:c.1A>G p.(Met1Val)	Smith-Lemli-Optiz syndrome
8	ERCC6	NM_000124.3:c.1685+5G>A p.?	Cockayne syndrome
	ERCC6	NM_000124.3:c.2167C>T p.(Gln723Ter)	Cockayne syndrome
9	GALT	NM_000155.3:c.563A>G p.(Gln188Arg)	Galactosaemia
	GALT	NM_000155.3:c.563A>G p.(Gln188Arg)	Galactosaemia
10	GJB2	NM_004004.5:c.109G>A p.(Val37Ile)	Deafness, autosomal recessive
	GJB2	NM_004004.5:c.109G>A p.(Val37Ile)	Deafness, autosomal recessive
11	PAH	NM_000277.1:c.1241A>G p.(Tyr414Cys)	Phenylketonuria
	PAH	NM_000277.1:c.527G>A p.(Arg176Gln)	Phenylketonuria
12	PAH	NM_000277.1:c.898G>T p.(Ala300Ser)	Phenylketonuria
	PAH	NM_000277.1:c.1222C>T p.(Arg408Trp)	Phenylketonuria
13	TREX1	NM_016381.4:c.790_793dupCAGT p.(Trp265SerfsTer32)	Aicardi-Goutières syndrome
	TREX1	NM_016381.4:c.401_408dupCTGCAGCC p.(Ser137LeufsTer9)	Aicardi-Goutières syndrome

Table 3. Thirteen carrier couples, genes, variants and disease associated with their variants

Thirteen carrier couples (table 3) were identified utilising this screening panel, each partner with a variant in the same gene (CBS, CFTR, DHCR7, ERCC6, GALT, GJB2, PAH and TREX1).
These carrier couples are at risk of having an affected child with the associated autosomal recessive disorder. Genetic counselling with pre-implantation genetic testing was available.

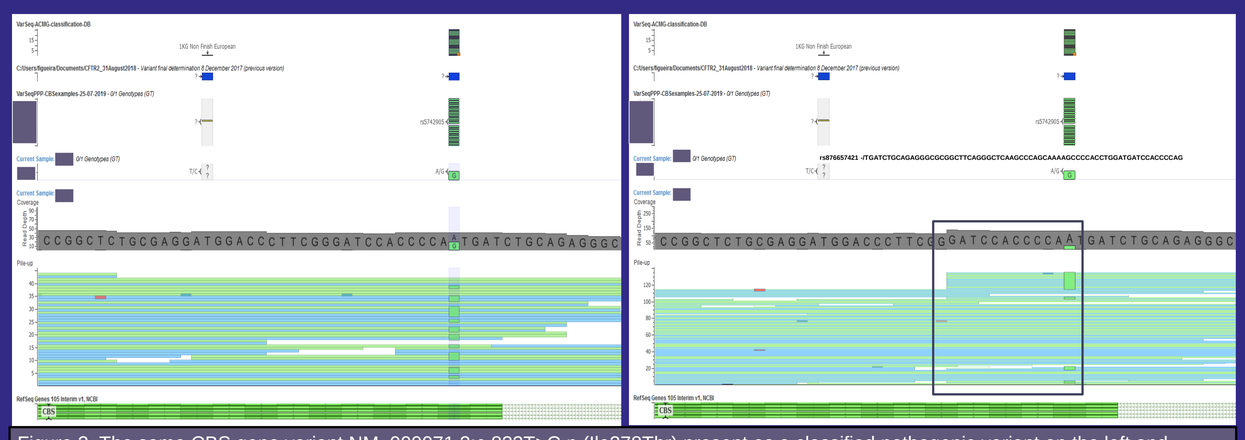

Figure 2. The same CBS gene variant NM_000071.2:c.833T>C p.(Ile278Thr) present as a classified pathogenic variant on the left and present as a benign variant on the right. The insertion creates a splice site, creating a new start to the exon and functionally removing the pathogenic variant.

Figure 2 shows a well documented and known pathogenic variant in the CBS gene. NM_000071.2:c.833T>C p.(Ile278Thr) may be reported incorrectly as pathogenic (as classified by ACMG guidelines/ClinVar) for homocystinuria. The insertion creates a splice acceptor site, creating a new start to the exon and removing the pathogenic variant in the functional transcript. In this scenario, even though the variant is detected by the software and is classified as pathogenic, each instance needs to be checked for the insertion 11bp downstream of the variant, and thus be correctly reported as a benign variant.

200 cross referenced couples (identified by the clinician before testing) were screened with no joint carrier risk i.e. no shared variants in the same gene. 180 donors were screened in total, with 73 being reported with no variants. Five patients, including 1 donor, had a total of 5 different variants reported (table 4).

Variants reported	1 Variant	2 Variants	3 Variants	4 Variants	5 Variants
Total number of patients	676	281	100	30	5
Percentage of patients	61.7	25.7	9.1	2.7	0.5

Table 4. Multiple variants reported in a cross section of patients

Interpreting and reporting any variant without a disease phenotype is a challenge. All data is analysed and stored in Australia. A comparison using Illumina's Nextera Flex[2] Library preparation was presented at the recent ASDG meeting in Adelaide. MiSeq and Sentieon alignment/variant calling are likewise being compared. Euformatics' omnomicsQ and omnomicsV[3] are used to monitor the quality of FASTQ, BAM and VCF data at any one time and over each time period. Illumina Variant Studio and Golden Helix[4] VarSeq with it's VSPipeline are compared. Alamut, gnomAD, ClinVar, HGMD Professional, Google Scholar, PubMed and other available resources are used to streamline the interpretative process. A local variant database is available using Golden Helix's VSWarehouse. CNV evaluation using VSCNV and reporting directly to the local laboratory information system using VSReport are expected to follow soon.

References:
1. Bell CJet al. (2011) Carrier Testing for Severe Childhood Recessive Diseases by Next-Generation Sequencing Sci Transl Med. 2011 Jan 12; 3(65): 65ra4.
2. Illumina [Internet]. Illumina Inc;2019. Nextera Flex for Enrichment Reference Guide; January 2019 [17 March 2019]. Available from: http://sapac.support.illumina.com/downloads/nextera-flex-for-enrichment-reference-guide-1000000048041.html
3. Euformatics omnomicsQ omnomicsV https://euformatics.com/
4. GoldenHelix VarSeq VSWarehouse Sentieon VSCNV VSReport https://www.goldenhelix.com/