Ending diagnostic odyssey using clinical whole-exome sequencing (CWES)

Abstract

**Objectives:** Most rare diseases are genetic diseases. Due to the diversity of rare diseases and the high likelihood of patients with rare diseases to be undiagnosed or misdiagnosed, it is not unusual that these patients undergo a long diagnostic odyssey before they receive a definitive diagnosis. This situation presents a clear need to set up a dedicated clinical service to end the diagnostic odyssey of patients with rare diseases.

**Methods:** Therefore, in 2014, we started an Undiagnosed Diseases Program in Hong Kong with the aim of ending the diagnostic odyssey of patients and families with rare diseases by clinical whole-exome sequencing (CWES), who have not received a definitive diagnosis after extensive investigation.

**Results:** In this program, we have shown that genetic diseases diagnosed by CWES were different from that using traditional approaches indicating that CWES is an essential tool to diagnose rare diseases and ending diagnostic odysseys. In addition, we identified several novel genes responsible for monogenic diseases. These include the **TOP2B** gene for autism spectrum disorder, the **DTYMK** gene for severe cerebral atrophy, the **KIF13A** gene for a new mosaic ectodermal syndrome associated with hypomelanosis of Ito, and the **CDC25B** gene for a new syndrome of cardiomyopathy and endocrinopathy.

**Conclusions:** With the incorporation of CWES in an Undiagnosed Diseases Program, we have ended diagnostic odysseys of patients with rare diseases in Hong Kong in the past 7 years. In this program, we have shown that CWES is an essential tool to end diagnostic odysseys. With the declining cost of next-generation sequencers and reagents, CWES set-ups are now affordable for clinical laboratories. Indeed, owing to the increasing availability of CWES and treatment modalities for rare diseases, precedence can be given to both common and rare medical conditions.

*Corresponding author: Ching-Wan Lam, Department of Pathology, The University of Hong Kong, Hong Kong, P.R. China, E-mail: ching-wanlam@pathology.hku.hk

Keywords: clinical whole exome sequencing; diagnostic odyssey; rare diseases.

Introduction

According to the World Health Organization, a rare disease is one that affects a small percentage of the population (i.e., 0.65–1 affected person out of 1,000 individuals). According to the Rare List (http://globalgenes.org/rarelist/), 300 million people worldwide have approximately 7,000 rare diseases. Patients with rare diseases typically go through a long diagnostic odyssey due to the challenges that accompany the diagnosis of rare diseases. Over the past 30 years, most of the patients with rare diseases referred to us for genetic testing were either undiagnosed or misdiagnosed. For example, we encountered a patient with Wilson disease, which is a treatable liver disease, who was undiagnosed for 18 years [1]. Another patient received a definitive diagnosis of citrin deficiency as the cause of neonatal jaundice till the age of 14 [2]. On a positive note, we ended the diagnostic odyssey of a family with three adults who have dysferlinopathy that was misdiagnosed as polymyositis and connective tissue diseases for more than 10 years [3]. Further, we identified a **POLG**-related mutation in a patient with mitochondrial recessive ataxia syndrome (includes SANDO and SCAE) misdiagnosed as mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS) [4]. The patient first presented at 8 years of age, and she finally received the correct diagnosis at 18 years of age. In a case such as this, a correct diagnosis can facilitate the provision of appropriate genetic counseling to the family (in this case, inheritance is autosomal recessive, not the maternal inheritance observed in MELAS). The correct diagnosis was beneficial for the patient and her family because they were advised to avoid valproic acid, which is a common anti-epileptic drug that can cause liver toxicity in **POLG**-carriers.

To end the diagnostic odyssey of patients with rare diseases, it is necessary to set up an Undiagnosed Disease Program. The program can provide important insights into the pathogenesis of rare diseases based on the novel gene–disease association. Guided management can then be provided to the patients, and the accuracy of prognosis...
prediction can be enhanced. In the same vein, genetic counseling can be more specific, recurrent risk calculation can be more accurate, and direct screening of complications for early detection and treatment can be initiated. With the advent of next generation sequencing (NGS) technology, many patients with undiagnosed diseases can now enjoy the benefits of a diagnostic tool such as clinical whole-exome sequencing (CWES). Since DNA extraction and generation of NGS libraries can be automated by liquid handlers, a NGS facility for molecular diagnosis can be managed by a single operator. Since de-multiplexing and alignment of NGS reads are performed during sequence runs and processing of data files for variant detection are automated by the sequencers, lack of expertise in bioinformatic analysis is no longer a barrier for the implementation of CWES in clinical laboratories. Taken together, advancements in NGS and bioinformatics technologies enabled the setting up Undiagnosed Disease Programs in clinical laboratories.

In 2014, we started the Undiagnosed Diseases Program in Hong Kong with the aim of ending the diagnostic odysseys of patients and families with rare diseases who do not have a definitive diagnosis after extensive investigation. In this program, we have shown that CWES is an essential tool to end diagnostic odysseys by the minimal overlaps of monogenic diseases diagnosed by CWES and non-CWES/traditional approaches in the past 30 years in the author’s laboratory.

Materials and methods

Patients were referred by clinicians specialized in cardiology, endocrinology, genetics, hepatology, metabolic medicine, nephrology, neurology, obstetrics, pathology, and pediatrics. Blood samples were collected from the patient and her family members after informed consent. Genomic DNA was extracted from whole blood samples by the QiAamp blood kit (Qiagen, Hilden, Germany) according to the manufacturer’s instruction. Clinical whole-exome sequencing (CWES) was performed using SureSelectXT Human All Exon V4 target kits (Agilent Technologies, Santa Clara, USA) and Nextera kits (Illumina, San Diego, USA) according to the manufacturers’ protocols. Sequencing was performed using an Illumina sequencer with 100-bp paired-end module (Illumina). Image analysis and base-calling were performed using the standard Illumina data analysis pipeline. CWES data filtering was performed using VariantStudio (version 2.2.1, Illumina). The in silico prediction of the damaging effects of each single-nucleotide variant were assessed using PolyPhen and SIFT. Variants were filtered based on population frequency, inheritance patterns, and in silico predictions. Resources such as the Human Gene Mutation Database, 1,000 Genomes database, Exome Aggregation Consortium, OMIM, PubMed, and ClinVar were used to evaluate the sequence variants of interest. Interpretation of variants was in accordance with the 2015 American College of Medical Genetics and Genomics standards and guidelines for the interpretation of sequence variants [5]. The pathogenic variants were described according to the Human Genome Variation Society guidelines on nomenclature for the description of sequence variants (http://www.hgvs.org/mutnomen).

Results and discussion

Through the Undiagnosed Disease Program, we were able to identify 42 monogenic diseases diagnosed by CWES and several of them are potentially treatable conditions [6–12] (Table 1). For instance, we identified a genetic defect in the thiamine pyrophosphokinase gene (TPKI) using CWES, thereby ending the 40-year diagnostic odyssey of a patient who presented with Leigh-like symptoms [6]. The TPKI gene is responsible for encoding thiamine pyrophosphokinase, an important enzyme in thiamine metabolism, and a genetic defect in the TPKI gene can lead to episodic encephalopathy. Thus, early dietary intervention/supplementation may reverse or slow down the disease progression. In contrast a total of 106 monogenic diseases were diagnosed by traditional/non-CWES methods in the past 30 years in the author’s laboratory [6, 13–116] (Table 1). Through a Venn diagram analysis, we showed that the spectrum of monogenic diseases diagnosed by CWES overlapped minimally with that of diseases diagnosed using non-CWES/traditional laboratory methods (Figure 1). Hence, it is more difficult to diagnose these diseases using clinical and traditional laboratory methods that do not involve CWES.

We also identified several novel genes for monogenic diseases using CWES. The genes include the TOP2B gene for autism spectrum disorder [7], the DYTMYK gene for severe cerebral atrophy [8], the KIF13A gene for a new ectodermal syndrome [9], and the CDC25B gene for a new syndrome of cardiomyopathy and endocrinopathy [10]. In the case of the TOP2B gene, the proband presented with global developmental delay and intellectual disability associated with a de novo TOP2B mutation. CWES of the proband revealed that she was heterozygous for NM_001068.2:c.172C>T; NP_001059.2:p.His58Tyr of the TOP2B gene. The mutation in the patient is a de novo mutation. TOP2B encodes for the enzyme topoisomerase II isoenzyme beta, which is abundant in the developing brain and in the adult brain. Three years after the publication of this case, an identical de novo variant was identified in a Japanese patient with a similar phenotype [117]. In the case of the CDC25B gene, the patient was an 11-year-old Chinese girl born to consanguineous asymptomatic parents with a history of one fetal death and one infant death, both of unknown causes. The proband suffered intrauterine
Table 1: Gene panels of diseases diagnosed by CWES and non-CWES/traditional methods. Only SUOX and ABCD1 are shared between the two panels.

(A) A gene panel of 42 diseases diagnosed by CWES

ABCD1, adrenoleukodystrophy; AK9, new syndrome; ATP8B1, cholestasis, progressive familial intrahepatic 1; BRAF, cardiofaciocutaneous syndrome; C3, C3 deficiency; CDC25B; new syndrome; COL12A1, Bethlem myopathy 2; COL4A5, Alport syndrome; COQ4, coenzyme Q10 deficiency, primary, 7; COX20, mitochondrial complex IV deficiency, nuclear type 11; CYP7B1, spastic paraplegia 5A, autosomal recessive; DDX3X, Intellectual developmental disorder, X-linked, syndrome, Snijders Blok type; DTM2, new syndrome; EBF3, hypopituitarism, ataxia, and delayed development syndrome; FBN1, Marfan syndrome; FDXR, auditory neuropathy and optic atrophy; FGFR3, hypochondroplasia; GNAO1, developmental and epileptic encephalopathy 17; GRIN2B, developmental and epileptic encephalopathy 27; GTPBP3, combined oxidative phosphorylation deficiency; IFIH1, Acarid–Gouïtères syndrome 7; KDM6A, Kabuki syndrome 2; KIF1A, NESVAC syndrome; KIF1A, new syndrome; KRT1, cerebral cavernous malformations; LCAM1, hydrocephalus due to aqueductal stenosis; LMBRD2, new syndrome; NR2F1, Bosch-Boonstra-Schaaf optic atrophy syndrome; PEX6, peroxisome biogenesis disorder 4A (Zellweger); PIGO, hyperphosphatasia with mental retardation syndrome 2a; PURA, neurodevelopmental disorder with neonatal respiratory insufficiency, hypopituitarism, and feeding difficulties; RAPSN, congenital myasthenic syndrome; RYR1, minicore myopathy with external ophthalmoplegia; SATB2, Glass syndrome; SERPINF1, osteogenesis imperfecta, type Vb; SLC16A2, Allan–Herndon–Dudley syndrome; SPTAN1, developmental and epileptic encephalopathy 5; SUOX, isolated sulfite oxidase deficiency; TBC1D24, developmental and epileptic encephalopathy 16; TOP2B, new syndrome; TPK1, thiamine metabolism dysfunction syndrome 5 (episodic encephalopathy type); WT1, Frasier syndrome.

(B) A gene panel of 106 diseases diagnosed by non-CWES/traditional methods

ABCB1, progressive familial intrahepatic cholestasis 2; ABCG8, hyperuricosuric hyperglycemia, familial 1; ABCD1, adrenoleukodystrophy; ABCG5, sitosterolemia; ACADM1, very long-chain acyl-coenzyme A dehydrogenase deficiency; ACTA1, nemaline myopathy 3; AGL, glycogen storage disease type II; AGKT, primary hyperoxaluria I; ALB, familial dysalbuminemic hyperlipoproteinemia; ARG1, arginase deficiency; APPL1, hypophosphatasia; AR, Kennedy syndrome; ARS8, Maroteaux–Lamy syndrome; ASL, arginosuccinate lyase deficiency; ATM, ataxia telangiectasia; ATP2A2, Darier disease; ATP2C1, Hailey–Hailey disease; ATP7B, Wilson disease; AVPR2, X-linked nephrogenic diabetes insipidus; BACH, butyrylcholinesterase deficiency; CASR, familial hypocalciuric hypercalcemia; CDC73, familial hyperparathyroidism; CHAT, myasthenic syndrome, congenital, 6, presynaptic; CLCN7, malignant infantile osteopetrosis; COL2A1, spondylophysis dysplasia congenita; COLQ, myoclonus-dystonia syndrome, congenital, 5; CPT2, carnitine palmitoyltransferase II deficiency; CYP17A1, steroid 17 alpha-hydroxylase deficiency; CYP21A2, adrenal hyperplasia, congenital, due to 21-hydroxylase deficiency; CYP27A1, cerebroretinoid xanthomatosis; DMPK, myotonia congenita; DPDY, dihydropyrimidine dehydrogenase deficiency; DYT1, primary torsion dystonia; FAH, tyrosinemia type 1; FCH, erythrocytic protoporphyria; FH, fumarate dehydratase-associated hereditary leukoencephalopathy and renal cell carcinoma syndrome; G6PC, glycogen storage disease type 1a; GAA, glycogen storage disease type II; GBA, Gaucher disease; GBE1, glycogen storage disease type III; GCH1, dopa-responsive dystonia; GLA, Fabry disease; GLB1, GM1 gangliosidosis; GLRA1, hyperkplexia; GLUT1, glutamate dehydrogenase deficiency; GPN2, molybdenum cofactor deficiency; GRHPR, primary hyperoxaluria II; HCOL1, holocarboxylase synthetase deficiency; HMBS, acute intermittent porphyria; HPRT1, Kelley–Seegmiller syndrome; HTT, Huntington disease; IDS, Hunter syndrome; IDUA, Hunter syndrome; JMD, isovaleric acidemia; KCN2, Long QT syndrome 6; KCNJ1, congenital Bartter syndrome type II; KCNJ1, Long QT syndrome type; KRT17, stafocystoma multiplex; LPL, lipoprotein lipase deficiency; MEC2P, Rett syndrome; MMACHC, methylmalonyl CoA mutase deficiency, methylmalonic aciduria and homocystinuria (cblC type); MOCS1, molybdenum cofactor deficiency; mtDNA, Kearns–Sayre syndrome; Leber hereditary optic neuropathy/Leigh syndrome/ mitochondrial encephalomyopathy with lactic acidosis and stroke syndrome/Pearson syndrome; MUT, methylmalonic aciduria, mut(0) type; MVK, mevalonic aciduria; NAGLU, mucopolysaccharidosis type IIa; NPHS1, congenital nephrotic syndrome type 1; NRB1, congenital adrenal hypoplasia; OTC, ornithine transcarbamylase deficiency; PANK2, pantothenate kinase 2 deficiency; PARK2, juvenile parkinsonism; PDCD6, congenital nephrotic syndrome type 2; PHKA2, glycogen storage disease type IX; POLG, mitochondrial respiratory deficiency (includes SANDO and SCAD); POMT1, muscular dystrophy–dystroglycanopathy (congenital with mental retardation), type B; PPOX, variegated porphyria; PROS1, protein S deficiency; PRTZ, episodic kinesinergic dyskinesia 1; PTC11, basal cell nevus syndrome; PTRN1, LOPED syndrome; PTS, 5-pyruvoyl-tetrahydrodopterin synthase deficiency; RYR2, catecholaminergic polymorphic ventricular tachycardia; SCN1A, Dravet syndrome; SCN4A, paramyotonia congenita; SDHD, succinate dehydrogenase deficiency; SLC12A1, antenatal Bartter syndrome type I; SLC12A3, Gitelman syndrome; SLC2A13, citrin deficiency; SLC2A5, hyperoxanthinemia–hyperammonemia–homocitrullinuria syndrome; SLC2A5, citrin–ammonium translocase deficiency; SLC2A6, Pendred syndrome; SLC37A4, glycogen storage disease type IIb; SLC3A4, cystinuria; SLC4A1, distal renal tubular acidosis; SLC7A9, cystinuria; SLC16A1, spinal muscular atrophy; SOD1, 5-alpha reductase deficiency; SUOX, isolated sulfite oxidase deficiency; TAZ, Barth syndrome; TH, thyrosine hydroxylase deficiency; THR, resistance to thyroid hormone syndrome; TTPA, classical late-infantile neuronal ceroid lipofuscinosis; TTR, familial amyloidotic polyneuropathy type 1; U1R1, hypoparicemia; VHL, von Hippel–Lindau syndrome; XPC, xeroderma pigmentosum type C.
growth retardation, delayed development, bilateral cataract (at 5 years of age), primary hypothyroidism, growth hormone deficiency, and cardiomyopathy (at 9 years of age). CWES of the proband was performed, and we identified a novel homozygous nonsense variant of the CDC25B gene known as NM_021873:c.313G>T (p.Glu105*). The c.313G>T in the proband was expected to produce a truncated protein that is terminated at codon 105 with a loss of phosphorylation sites. We posit that a loss-of-function CDC25B gene will result in a new syndrome characterized by cataract, dilated cardiomyopathy, and multiple endocrinopathies. In the case of the DTYMK gene, the patients were two brothers aged one and 6 years who presented with microcephaly, marked cerebral atrophy with bilateral subdural hemorrhagic effusion, hypotonia, severe intellectual disability, and lactic acidosis. Two mutations in the DTYMK gene were identified in the brothers. The first mutation is the frameshift mutation NM_012145.3:c.287_320del;p.Asp96-Valfs*8 and the second one is the missense mutation NM_012145.3:c.295G>A;p.Ala99Thr. In the case of the KIF13A gene, the patient was a three-year-old girl who presented with developmental delay and hypomelanosis of Ito. An exome-and-genome approach revealed a heterozygous de novo frameshift variant in the KIF13A gene (i.e., NM_022113.6: c.2357dupA). The low mutant allelic ratio suggested that the mutation occurred postzygotically, leading to embryonic mosaicism. We suggest KIF13A may be the culprit gene in chr 6p22.3–p23 microdeletion syndrome because skin hypopigmentation has been reported in patients with the syndrome [118].

Figure 1: A Venn diagram of gene panels of diseases diagnosed by CWES and non-CWES/traditional methods in the author’s laboratory in the past 30 years. CWES group: a gene panel of 42 diseases diagnosed by CWES; Traditional group: A gene panel of 106 diseases diagnosed by non-CWES/traditional methods. The gene lists are shown in Table 1.

Conclusions

With the incorporation of CWES in an Undiagnosed Diseases Program, we have ended diagnostic odysseys of patients with rare diseases in Hong Kong in the past 7 years. With the technological advancement of NGS, CWES can now be completed in a clinical laboratory in 2 days. In addition, with the declining cost of next-generation sequencers and reagents, CWES set-ups are now affordable for clinical laboratories and precedence can be given to both common and rare medical conditions.

Acknowledgments: I would like to thank all the patients, and families who participated in this program.

Research funding: This work was supported by S.K. Yee Medical Foundation Grant (no. 2141219) – Undiagnosed Diseases Program for ending diagnostic odyssey.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors’ institutional review board (UW21-150).

References

1. Mak C, Tam S, Fan ST, Liu CL, Lam CW. Wilson’s disease: a case that escaped diagnosis for eighteen years. Hong Kong Med J 2006;12:154–8.
2. Lee HC, Lam CW, Yuen YP, Lai CK, Chan KY, Chan AY. In search of the diagnosis, and the patient: transient galactosemia demystified after 14 years. Genet Counsel 2012;23:415–21.
3. Lau KC, Mak CM, Leung KY, Tsoi TH, Tang HY, Lee P, et al. A fast modified protocol for random-access ultra-high-density whole-genome scan: a tool for personalized genomic medicine, positional mapping, and cytogenetic analysis. Clin Chim Acta 2009;406:31–5.
4. Lam CW, Law CY, Siu WK, Fung CW, Yau MM, Huen KF, et al. Novel POLG mutation in a patient with sensory ataxia, neuropathy, ophthalmoparesis and stroke. Clin Chim Acta 2015;448:211–4.
5. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. ACMG Laboratory Quality Assurance Committee. 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.
6. Au LWC, Lee HHC, Sheng B, Chan KY, Yau EKC, Mak CM, et al. Movement disorders associated with thiamine pyrophosphokinase deficiency: intrafamilial variability in the phenotype. Clin Neurol Neurosurg 2020;199:106258.

7. Lam CW, Yeung WL, Law CY. Global developmental delay and intellectual disability associated with a de novo TOP2B mutation. Clin Chim Acta 2017;469:63–8.

8. Lam CW, Yeung WL, Ling TK, Wong KC, Law CY. Deoxythymidylate kinase, DTYMK, is a novel gene for mitochondrial DNA depletion syndrome. Clin Chim Acta 2019;496:93–9.

9. Lam CW, Chan CY, Wong KC, Chang ST. Postzygotic inactivating mutation of KIF13A located at chromosome 6p22.3 in a patient with a novel mosaic neuroectodermal syndrome. J Hum Genet 2021;66:825–9.

10. Lam CW, Fong NC, Chan TY, Lau KC, Ling TK, Mak DW, et al. Centrosome-associated CDC25B is a novel disease-causing gene for a syndrome with cataracts, dilated cardiomyopathy, and multiple endocrinopathies. Clin Chim Acta 2020;504:81–7.

11. Ling TK, Law CY, Yan KW, Fong NC, Wong KC, Lee KL, et al. Clinical whole-exome sequencing reveals a common pathogenic variant in patients with CoQ10 deficiency: an underdiagnosed cause of mitochondrialopathy. Clin Chim Acta 2019;497:88–94.

12. Lam CW, Wong KS, Leung HW, Law CY. Limb girdle myasthenia with digenic RAPSN and a novel disease gene AK9 mutations. Eur J Hum Genet 2017;25:192–9.

13. Lam CW, Law CY, Leung KF, Lai PK, Pak-lam Chen S, Chan B, et al. NMR-based urinalysis for rapid diagnosis of β-ureidopropionase deficiency in a patient with Dravet syndrome. Clin Chim Acta 2015;440:201–4.

14. Law CY, Chang ST, Cho SY, Yau EK, Ng GS, Fong NC, et al. Clinical whole-exome sequencing reveals a novel missense pathogenic variant of GNAO1 in a patient with infantile-onset epilepsy. Clin Chim Acta 2015;451:292–6.

15. Law CY, Yeung WL, Cheung YF, Chan HF, Fung E, Hui J, et al. A common PRRT2 mutation in familial paroxysmal kinesigenic dyskinesia in Hong Kong: a case series of 16 patients. Hong Kong Med J 2016;22:619–22.

16. Lai CK, Lam CW, Chan YW. High performance thin-layer chromatography of free porphyrins for diagnosis of porphyria. Clin Chim Acta 1994;40:2026–9.

17. Lam CW, Jain K, Chan KY, Silva DK, Chan YW, Wong LJC. Diagnosis of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes in a Chinese family by PCR/restriction enzyme analysis. J Clin Path Mol Pathol 1995;48:M285–8.

18. Lam CW, Lau CH, Williams JC, Chan YW, Wong LJC. Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes (MELAS) triggered by valproate therapy. Eur J Pediatr 1997;156:562–4.

19. Yuen WF, Lam CW, Chow TC, Chiu MC. A characteristic dissection microscopy appearance of a renal biopsy of a Fabry heterozygote. Nephron 1997;77:394–6.

20. Wong LJC, Lam CW. Alternative, non-invasive tissues for quantitative screening of mutant mitochondrial DNA. Clin Chem 1997;43:1241–3.

21. Lam CW, Lai CK, Chan YW. Simultaneous fluorescence detection of fecal urobilins and porphyrins by reversed-phase high-performance thin-layer chromatography. Clin Chem 1998;44:345–6.

22. Lam CW, But WM, Shek CC, Tong SF, Chan YS, Choy KW, et al. Glucose-6-phosphatase gene (727G→T) splicing mutation is prevalent in Hong Kong Chinese patients with glycogen storage disease type 1a. Clin Genet 1998;53:184–90.

23. Lam CW, Tong SF, Lam YY, Chan BY, Ma CH, Lim PL. Identification of a novel missense mutation (G149E) in glucose-6-phosphate translocase gene in a Chinese family with glycogen storage disease 1b. Hum Mutat 1999;13:507.

24. Orrico A, Lam CW, Galli L, Dotii MT, Hayek G, Tong SF, et al. MECP2 mutation in male patients with non-specific X-linked mental retardation. FEBS Lett 2000;481:285–8.

25. Lee V, Li CK, Shing MMK, Chik KW, Lam CW, Tsang KS, et al. Umbilical cord blood transplantation for Maroteaux–Lamy syndrome (mucopolysaccharidosis VI). Bone Marrow Transplant 2000;26:455–8.

26. Lam CW, Sin SY, Lau E, Lam YY, Poon P, Tong SF. Prenatal diagnosis of glycogen storage disease type 1b using denaturing high-performance liquid chromatography. Prenat Diagn 2000;20:765–8.

27. Lam CW, Chan KY, Tong SF, Chan BY, Chan YT, Chan YW, et al. A novel missense mutation (P191L) in the glucose-6-phosphate translocase gene identified in a Chinese family with glycogen storage disease 1b. Hum Mutat 2000;16:94.

28. Lam CW, Mak YT, Lo YMD, Tong SF, To KF, Lai FMM. Molecular genetic analysis of a Chinese patient with Fabry disease. Chin Med J 2000;113:186–8.

29. Lam CW, Arlt W, Chan CK, Honour JW, Lin CJ, Tong SF, et al. Mutation of proline 409 to arginine in the meander region of cytchrome P450c17 causes severe 17α-hydroxylase deficiency. Mol Genet Metabol 2001;72:254–9.

30. Lam CW, Hui KN, Poon PMK, Luk NM, Yuen YP, Tong SF, et al. Novel splicing mutation of the PPOX gene (IVS10 +1G→A) detected by denaturing high-performance liquid chromatography. Clin Chim Acta 2001;197–200. https://doi.org/10.1016/S0009-8981(00)00382-5.

31. Lam CW, Poon PMK, Tong SF, Lo AWI, Lai CK, Choi KL, et al. Novel mutation and polymorphisms of the HMBS gene detected by denaturing HPLC. Clin Chem 2001;47:343–6.

32. Lam CW, Ko CH, Poon PMK, Tong SF. Two novel CLN2 gene mutations in a Chinese patient with classical late-infantile neuronal ceroid lipofuscinosis. Am J Med Genet 2001;99:161–3.

33. Ko CH, Lam CW, Tse PWT, Kong CK, Chan AKH, Wong LC. De novo mutation in the mitochondrial IRNA Leu (UUR) gene (A3243G) with rapid segregation resulting in MELAS in the offspring. J Paediatr Child Health 2001;37:89–90.

34. Chow KM, Hui CF, Lam CW, Morgan RR, Whatley SD, Kay R, et al. Clinical and genetic features of variegate porphyria in a Chinese patient. Chin Med J 2001;114:424–7.

35. Kong CK, Lam CW, Tse PWT, Kong CK, Chan AKH, Wong LC. A novel missense mutation (P191L) in the glucose-6-phosphate translocase gene identified in a Chinese family with glycogen storage disease 1b. Mol Genet Metabol 2000;26:455–8.

36. Lam CW, Yuen YP, Lai CK, Tong SF, Chan KY, Ng GS, et al. DNA-based diagnosis of isolated sulphite oxidase deficiency by denaturing high-performance liquid chromatography. Mol Genet Metabol 2002;75:91–5.
39. Lam CW, Cheng AWF, Tong SF, ChanYW. Novel donor splice site mutation of ABCG5 gene in sitosterolemia. Mol Genet Metabol 2002;75:178–80.
40. Dotti MT, Orrico A, De Stefano N, Battisti C, Sicurelli F, Severi S, et al. A Rett syndrome MECP2 mutation that causes mental retardation in men. Neurology 2002;58:226–30.
41. Lam CW, Leung CY, Lee KC, Xie J, Lo FM, Au TS, et al. Novel mutations in the PATCHED gene in basal cell nevus syndrome. Mol Genet Metabol 2002;76:57–61.
42. Yuen YP, Cheng WF, Tong SF, Chan YT, ChanYW, Lam CW. Novel missense mutation (Y249H) in the G6P1 gene causing glycogen storage disease type 1b. Mol Genet Metabol 2002;77:249–51.
43. Chan LYS, Lam CW, Mak YT, Tomlinson B, Tsang MW, Baum L, et al. Genotype-phenotype studies of six novel LPL mutations in Chinese patients with hypertriglyceridemia. Hum Mutat 2002;20:232–3.
44. Mak CM, Lam CW, Fan ST, Liu CL, Tam SC. Genetics of familial amyloidotic polyneuropathy in a Hong Kong Chinese kindred. Acta Neurol Scand 2003;107:419–23.
45. Au KM, Lai CK, Yuen YP, Shek CC, Lam CW, Chan AYW. Diagnosis of dihydropyrimidine dehydrogenase deficiency in a neonate with thymine-uraciluria in Hong Kong. Hong Kong Med J 2003;9:130–2.
46. Lam CW, Yuen YP, Chan KY, Tong SF, Lai CK, Chow TC, et al. Juvenile-onset glycogen storage disease type II with novel mutations in acid a-glucosidase gene. Neurology 2003;60:715–7.
47. Lam CW, Lai CK, Chow CB, Tong SF, Yuen YP, Mak YF, et al. Ethnic-specific splicing mutation of the carnitine-acylcarnitine translocase gene in a Chinese neonate presenting with sudden unexpected death. Chin Med J 2003;116:1110–2.
48. Lam CW, Lo IFM, Tong SF, LamSTS. Novel mutation of the PATCHED gene in a patient with basal cell nevus syndrome and teratology of Fallot. HK Dermato Venerol Bull 2003;11:4–8.
49. Yang T, Pang CP, Tsang MW, Lam CW, Poon PM, Chan LY, et al. Pathogenic mutations of the lipoprotein lipase gene in Chinese patients with hypertriglyceridemic type 2 diabetes. Hum Mutat 2003;21:453.
50. Yuen YP, Lai CK, Tong GMW, P Wong PM, Wong FKM, Mak SK, et al. Novel mutations of the AGXT gene causing primary hyperoxaluria type 1. J Nephrol 2004;17:436–40.
51. Lee CY, Lam CW, Shek CC. Steroid 5 alpha-reductase 2 deficiency in two generations of a non-consanguineous Chinese family. J Pediatr Endocrinol Metab 2003;16:1197–201.
52. Li CH, Lam CW, Lee ACW, Kwong NS, Szeto SC. Pearson’s syndrome: a rare cause of non-immune hydrops fetalis. Chin Med J 2003;116:1952–4.
53. Chan AOK, Lam CW, Tong SF, Tung CM, Yung K, ChanYW, et al. Novel mutations in the BCHE gene in patients with no butyrylcholinesterase activity. Clin Chir Acta 2005;351:155–9.
54. Lam CW, Lee ATC, Lam YY, Wong TW, Mak TWL, Fung WC, et al. DNA-based subtyping of glycogen storage disease type III: mutation and haplotype analysis of the AGL gene in Chinese. Mol Genet Metabol 2004;83:271–5.
55. Lam CW, Chan AOK, Lai CK, Chan WH, ChanYW, Shek CC, et al. A novel mutation, Y259X, of the ARSB gene in a Chinese family with mucopolysaccharidosis type VI. Chin Med J 2004;117:1850–2.
56. Lam CW, Cheung KK, Luk NM, Chan SW, Lo KK, Tong SF. DNA-based diagnosis of xeroderma pigmentosum group C by whole-genome scan using single-nucleotide polymorphism microarray. J Invest Dermatol 2005;124:87–91.
57. Kong APS, Lam CW, Chan AOK, Yiu SF, Tiu SC. Resistance to thyroid hormone in a Chinese family with R429Q mutation in the thyroid hormone receptor beta gene. Hong Kong Med J 2005;11:125–9.
58. Lam CW, Chan AOK, Tong SF, Shek CC, Tiu SC. DNA-based diagnosis of thyroid hormone resistance syndrome: a novel THR8 mutation associated with mild resistance to thyroid hormone. Clin Chir Acta 2005;358:55–9.
59. Lam CW, Lee KF, Chan AOK, Poon PM, Law TY, Tong SF. Novel missense mutation in the CASR gene in a Chinese family with familial hypocalciuric hypercalcemia. Clin Chir Acta 2005;360:167–72.
60. Yeung WL, Lam CW, Cheng WT, Sin NC, Wong WK, Wong CN, et al. Early-onset primary torsional dystonia in a 4-generation Chinese family with a mutation in the DYT1 gene. Chin Med J 2005;118:873–6.
61. Yeung WL, Lam CW, Hui J, Tong SF, Wu SP. Galactorrhea—a strong clinical clue towards the diagnosis of neurotransmitter disease. Brain Dev 2006;28:389–91.
62. Lam CW, Yuen YP, Cheng WF, Chan YY, Tong SF. Missense mutation Leu72Pro located on the carboxyl terminal amphipathic helix of apolipoprotein C-II causes familial chylomiconemia syndrome. Clin Chir Acta 2006;364:256–9.
63. Wong VCN, Lam CW, Fung CW. Stiff child syndrome with mutation of DYT1 gene. Neurology 2006;65:1465–6.
64. Poon WT, Chan KY, Au KM, Tong SF, ChanYW, Lam CW, et al. Novel missense mutation (Y279S) in the GLRA1 gene causing hyperekplexia. Clin Chir Acta 2006;364:361–2.
65. Yuen YP, Lam CW, Lai CK, Tong SF, Li PS, Tam S, et al. Heterogeneous mutations in the SLC3A1 and SLC7A9 genes in Chinese patients with cystinuria. Kidney Int 2006;69:123–8.
66. Lam CW, Cheung KM, Tsui MS, Yan MSC, Lee CY, Tong SF. A patient with novel ABCB11 gene mutations with phenotypic transition between BRIC2 and PFIC2. J Hepatol 2006;44:240–2.
67. Lam CW, Mak CM. Allele dropout in PCR-based diagnosis of Wilson disease: mechanisms and solutions. Clin Chem 2006;52:517–20.
68. Lam CW, Orrico A, Yan MS, Law TY, Galli L, Benedetti A, et al. Resequencing the G6P1 gene reveals a novel splicing mutation in a patient with glycogen storage disease type 1b. Clin Chir Acta 2006;374:147–8.
69. Lam CW, Cheng WF, Yuen YP, Tong SF, Huen PF. Novel mutation, 1234delA, in the DAX1 gene in congenital adrenal hypoplasia. Clin Chir Acta 2006;364:256–9.
70. Basheer SN, Waters PJ, Lam CW, Acquaviva-Bourdin C, Henderson G, Poskitt K, et al. Isolated sulfite oxidase deficiency in the newborn: lactide acidemia and leuкоencephalopathy. Neuropediatrics 2007;38:38–41.
71. Lau KC, Lam CW. Molecular investigations of a novel iduronate-2-sulfatase mutant in a Chinese patient. Clin Chir Acta 2008;392:8–10.
72. Chan KY, Lam CW, Lee LP, Tong SF, Yuen YP. Pantothenate kinase-associated neurodegeneration in two Chinese children: identification of a novel PANK2 gene mutation. Hong Kong Med J 2008;14:70–3.
73. Mak CM, Lam CW, Tam S, Lai CL, Chan LY, Fan ST, et al. Mutational analysis of 65 Wilson disease patients in Hong Kong
Chinese: identification of 17 novel mutations and its genetic heterogeneity. J Hum Genet 2008;53:55–63.
74. Mak CM, Kwong YL, Lam CW, Chan SC, Lo CM, Fan ST, et al. Identification of a novel TRR Gly67Glu mutant and the first case series of familial transthyretin amyloidosis in Hong Kong Chinese. Amyloid 2007;14:293–7.
75. Mak CM, Siu TS, Lam CW, Chan GC, Poon GW, Wong KY, et al. Complete recovery from acute encephalopathy of late-onset ornithine transcarbamylase deficiency in a 3-year-old boy. J Inher Metab Dis 2007;30:981.
76. Ma RC, Lam CW, Chan WB, So WY, Tong SF, Chow CC, et al. A Chinese family with familial paraganglioma syndrome due to succinate dehydrogenase deficiency. Hong Kong Med J 2007;13:151–4.
77. Lam CW, Ng KF, Chan HM, Lee KP, Siu TS, Tam S. A novel mutation at a ligand-binding site of hypoxanthine-guanine phosphoribosyltransferase, p.Y105C (HPRT Hong Kong), in a Chinese teenager with recurrent gouty arthritis. Clin Chim Acta 2007;380:252–3.
78. Lee RS, Lam CW, Lai CK, Yuen YP, Chan KY, Shek CC, et al. Carnitine-acylcarnitine translocase deficiency in three neonates presenting with rapid deterioration and cardiac arrest. Hong Kong Med J 2007;13:66–8.
79. Mok NS, Lam CW, Fong NC, Hui YW, Choi YC, Chan KY. Cardiac ryanodine receptor gene (hRyR2) mutation underlyng catecholaminergic polymorphic ventricular tachycardia in a Chinese adolescent presenting with sudden cardiac arrest and cardiac syncope. Chin Med J (Engl) 2006;119:2129–33.
80. Lam CW, Yan MS, Li CK, Lau KC, Tong SF, Tang HY. DNA-based diagnosis of mucolipidosis type IIA and mucopolysaccharidosis type VI in a Chinese family: a chance of 1 in 7.6 trillion. Clin Chim Acta 2007;376:250–2.
81. Lam CW, Tong SF, Wong K, Luo YF, Tang HY, Ha SY, et al. DNA-based diagnosis of malignant osteopetrosis by whole-genome scan using a single-nucleotide polymorphism microarray: standardization of molecular investigations of genetic diseases due to consanguinity. J Hum Genet 2007;52:98–101.
82. Yuen YP, Lai CK, Chan YW, Lam CW, Tong SF, Chan KY. DNA-based diagnosis of methylenononic aciduria and homocystinuria, cblC type in a Chinese patient presenting with mild developmental delay. Clin Chim Acta 2007;375:171–2.
83. Lau KC, Lam CW. Automated imaging of circulating fluorescent for the diagnosis of erythropoietic protoporphyria: a pilot study for population screening. J Med Screen 2008;15:199–203.
84. Lau KC, Lam CW, Fong B, Siu TS, Tam S. DNA-based diagnosis of erythropoietic protoporphyria in two families and the frequency of a low-expression FECH allele in a Chinese population. Clin Chim Acta 2009;400:132–4.
85. Chik KK, Chan CW, Lam CW, Ng KL. Hyperinsulinism and hyperammonaemia syndrome due to a novel missense mutation in the allosteric domain of the glutamate dehydrogenase 1 gene. J Paediatr Child Health 2008;44:517–9.
86. Yeung WL, Lam CW, Fung LW, Hon KL, Ng PC. Severe congenital myasthenia gravis of the presynaptic type with choline acetyltransferase mutation in a Chinese infant with respiratory failure. Neonatology 2009;95:183–6.
87. Lam CW, Kong AP, Tsui TK, Ozaki R, Chan HM, Tong SF, et al. A novel mutation of SLC22A12 gene causing primary renal hypouricemia in a patient with metabolic syndrome. Clin Chim Acta 2008;398:157–8.
88. Lam CW, Tong SF, Wong K, Luo YF, Tang HY, Ha SY, et al. DNA-based diagnosis of malignant osteopetrosis by whole-genome scan using a single-nucleotide polymorphism microarray: standardization of molecular investigations of genetic diseases due to consanguinity. J Hum Genet 2007;52:98–101.
89. Lam CW, Kong AP, Tsui TK, Ozaki R, Chan HM, Tong SF, et al. A novel mutation of SLC22A12 gene causing primary renal hypouricemia in a patient with metabolic syndrome. Clin Chim Acta 2008;398:157–8.
90. Funayama M, Li Y, Tsai TH, Lam CW, Ohi T, Yazawa S, et al. Familial Parkinsonism with digenic parkin and PINK1 mutations. Mov Disord 2008;23:1461–5.
105. Siu WK, Law CY, Lam CW, Mak CM, Wong GW, Ho AY, et al. Novel nonsense CDC73 mutations in Chinese patients with parathyroid tumors. Fam Cancer 2011;10:695–9.

106. Mak CM, Lam CW, Fong NC, Siu WK, Lee HC, Siu TS, et al. Fatal viral infection-associated encephalopathy in two Chinese boys: a genetically determined risk factor of thermolabile carnitine palmitoyltransferase II variants. J Hum Genet 2011;56:617–21.

107. Lee HC, Mak CM, Lam CW, Yuen YP, Chan AO, Shek CC, et al. Analysis of inborn errors of metabolism: disease spectrum for expanded newborn screening in Hong Kong. Chin Med J (Engl) 2011;124:983–9.

108. Tsang JP, Poon WL, Luk HM, Fung CW, Ching CK, Mak CM, et al. Arginase deficiency with new phenotype and a novel mutation: contemporary summary. Pediatr Neurol 2012;47:263–9.

109. Siu WK, Mak CM, Siu SL, Siu TS, Pang CY, Lam CW, et al. Molecular diagnosis for a fatal case of very long-chain acyl-CoA dehydrogenase deficiency in Hong Kong Chinese with a novel mutation: a preventable death by newborn screening. Diagn Mol Pathol 2012;21:184–7.

110. Mak CM, Lee CY, Lam CW, Siu WK, Hung VC, Chan AY. Personalized medicine switching from insulin to sulfonylurea in permanent neonatal diabetes mellitus dictated by a novel activating ABCC8 mutation. Diagn Mol Pathol 2012;21:56–9.

111. Lee HC, Lai CK, Yau KC, Siu TS, Mak CM, Yuen YP, et al. Non-invasive urinary screening for aromatic L-amino acid decarboxylase deficiency in high-prevalence areas: a pilot study. Clin Chim Acta 2012;413:126–30.

112. Cho SY, Lau EY, Luk DC, Law CY, Lai CK, Lam CW. Novel PPOX exonic mutation inducing aberrant splicing in a patient with homozygous variegate porphyria. Clin Chim Acta 2021;512:117–20.

113. Tong CC, Lam CW, Lam KO, Lee VHF, Luk MY. A novel DPYD variant Associated with Severe toxicity of fluoropyrimidines: role of preemptive DPYD genotype Screening. Front Oncol 2018;8:279.

114. Chong YK, Ma LC, Lo KL, Lee CK, Mak CM, Kan AN, et al. Dystroglycanopathy with two novel POMT1 mutations in a Chinese boy with developmental delay and muscular dystrophy. Eur J Paediatr Neurol 2014;18:532–5.

115. Cho SY, Law CY, Ng KL, Lam CW. Novel large deletion in AVPR2 gene causing copy number variation in a patient with X-linked nephrogenic diabetes insipidus. Clin Chim Acta 2016;455:84–6.

116. Mak CM, Lam CW, Chim S, Siu TS, Ng KF, Tam S. Biochemical and molecular diagnosis of tyrosinemia type I with two novel FAH mutations in a Hong Kong Chinese patient: recommendation for expanded newborn screening in Hong Kong. Clin Biochem 2013;46:155–9.

117. Hiraide T, Watanabe S, Matsubayashi T, Yanagi K, Nakashima M, Ogata T, et al. A de novo TOP2B variant associated with global developmental delay and autism spectrum disorder. Mol Genet Genomic Med 2020;8:e1145.

118. Celestino-Soper PB, Skinner C, Schroer R, Eng P, Shenai J, Nowaczysk MM, et al. Deletions in chromosome 6p22.3–p24.3, including ATXN1, are associated with developmental delay and autism spectrum disorders. Mol Cytogenet 2012;5:17.