

ORIGINAL ARTICLE

Non-invasive prenatal diagnosis of achondroplasia and thanatophoric dysplasia: next-generation sequencing allows for a safer, more accurate, and comprehensive approach

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ABSTRACT

Objective Accurate prenatal diagnosis of genetic conditions can be challenging and usually requires invasive testing. Here, we demonstrate the potential of next-generation sequencing (NGS) for the analysis of cell-free DNA in maternal blood to transform prenatal diagnosis of monogenic disorders.

Methods Analysis of cell-free DNA using a PCR and restriction enzyme digest (PCR-RED) was compared with a novel NGS assay in pregnancies at risk of achondroplasia and thanatophoric dysplasia.

Results PCR-RED was performed in 72 cases and was correct in 88.6%, inconclusive in 7% with one false negative. NGS was performed in 47 cases and was accurate in 96.2% with no inconclusives. Both approaches were used in 27 cases, with NGS giving the correct result in the two cases inconclusive with PCR-RED.

Conclusion NGS provides an accurate, flexible approach to non-invasive prenatal diagnosis of *de novo* and paternally inherited mutations. It is more sensitive than PCR-RED and is ideal when screening a gene with multiple potential pathogenic mutations. These findings highlight the value of NGS in the development of non-invasive prenatal diagnosis for other monogenic disorders. © 2015 The Authors. *Prenatal Diagnosis* published by John Wiley & Sons, Ltd.

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INTRODUCTION

Prenatal diagnosis of monogenic disorders, including those presenting with ultrasound abnormalities, traditionally required analysis of fetal material obtained following invasive procedures such as amniocentesis and chorionic villus sampling, both of which carry a small but significant miscarriage risk.¹ The discovery of cell-free fetal DNA (cffDNA) circulating in maternal blood² is allowing the development of early non-invasive prenatal diagnosis (NIPD) based on analysis of maternal blood,³ eliminating the risk of directly causing fetal loss from invasive testing, which can only be performed after 11 weeks' gestation.¹ Prenatal testing for Down syndrome and other aneuploidies using cell-free DNA (cfDNA) is now widely available in the USA, Asia, and Europe, implementation being based on next-generation DNA sequencing [next-generation sequencing (NGS)] technology⁴ and driven by commercial providers.⁵ However, in the UK during 2011–2012, more than

2000 invasive tests were performed because of a high risk for a monogenic disorder.⁶ While there are many single case reports and the occasional small series reporting NIPD for single gene disorders, there has been little by way of implementation into routine clinical practice.⁷ In our National Health Service (NHS) Regional Genetics Service, we have been offering fetal sex determination based on the analysis of cffDNA for several years⁸ and, in 2012, obtained UK Genetic Testing Network approvals to deliver a diagnostic service based on the analysis of cffDNA using a PCR-based method for achondroplasia⁹ and thanatophoric dysplasia (TD).¹⁰

Achondroplasia is the most common non-lethal skeletal dysplasia with an incidence of ~5–15 per 100 000 live births. It is an autosomal dominant disorder, and ~98% of cases are caused by a c.1138G>A mutation in the fibroblast growth factor receptor 3 (*FGFR3*) gene,^{11,12} with ~1% caused by a c.1138G>C mutation.¹² Most cases arise *de novo* with no prior

familial history of skeletal dysplasia¹³ and present late in pregnancy when short limbs are detected by ultrasound scanning.⁹ TD is the most common lethal skeletal dysplasia, also resulting from *FGFR3* mutations,¹⁵ but presents earlier in pregnancy with short limbs, a small chest, and other features.¹⁰ Unlike achondroplasia, TD arises from several *FGFR3* mutations¹⁵ and can be separated radiologically into two forms, TD I and TD II with features that overlap. TD I has curved femora with infrequent craniosynostosis, whereas TD II is characterized by straight femora and a clover-shaped skull. A number of *FGFR3* mutations cause TD I, with the c.742C>T mutation accounting for more than 50% of cases. All cases of TD II are caused by a single c.1948A>G *FGFR3* mutation.¹⁵

The early PCR-based methods developed for NIPD of single gene disorders are suitable for the detection of paternally inherited alleles and can be useful in conditions such as achondroplasia where there is a single common causative mutation.^{7,11} In conditions such as TD, while this approach may be suitable to exclude recurrence, albeit the risk is very low, of a known mutation in families with a relevant family history, it is less useful for the diagnosis of ultrasound abnormalities in cases arising *de novo* when there are multiple potential causative mutations. Furthermore, as the majority of cfDNA is maternal in origin,¹⁶ simple molecular techniques such as PCR-RED may not be sufficiently sensitive for the delivery of accurate results in all cases.

Here, we describe the use of NGS to improve the accuracy and scope of NIPD of monogenic disorders using skeletal dysplasias as an example. We compare the results obtained using NGS with PCR-RED and demonstrate for the first time how, in clinical practice, NGS has the potential to transform prenatal diagnosis for some families at high risk for genetic disorders.

METHODS

Patient recruitment

The study included a mix of retrospective cases, ascertained by searching our nationally accredited Regional Genetics Laboratory records to identify all cases where cfDNA in maternal blood had been analyzed for an *FGFR3* mutation and cases presenting prospectively. In all cases, blood samples were collected from women at risk of carrying a fetus with achondroplasia or TD either because of sonographic findings or because of a relevant past family history. Where appropriate, written informed consent was obtained prior to venepuncture, and the study was approved by the University College London Hospital Ethics Committee A (reference 01/0095). In all cases, confirmation of prenatal testing result was sought from postnatal molecular testing, radiology, or pathology.

Testing using PCR-RED was available in our laboratory from 2007. Where there was sufficient DNA or frozen maternal plasma, available tests were repeated using the NGS panel. For cases tested prospectively, if sufficient plasma was available, PCR-RED and NGS analyses were run concurrently. Registered clinical scientists with at least 3 years' experience performed the tests and were blinded to clinical information and previous laboratory results.

Sample processing and DNA extraction

Plasma was separated from 20 mL of blood within 48 h of blood draw and cfDNA extracted using the QIAamp MinElute Virus Spin Kit (Qiagen) or QIAamp Circulating Nucleic Acid kit (Qiagen).^{8–10}

PCR-RED for *FGFR3* mutations

PCR-RED for the diagnosis of achondroplasia and TD was carried out as previously described.^{9,10} Assays were designed for the common c.1138G>A achondroplasia mutation and two of the TD mutations, the 1948A>G mutation that causes TD II and the most common TD I mutation, c.742C>T.

FGFR3 – next-generation DNA sequencing assay

Five amplicons covering 29 known disease-causing mutations in the *FGFR3* gene (Table 1) were designed using Primer 3 software.¹⁷ Our custom design for the PCR primers included the adapters and index sequences. The panel was validated using normal and positive control genomic DNA samples obtained by chorionic villus sampling to ensure that the panel was able to detect the expected mutations.

DNA sequencing library preparation

PCR was carried out on plasma cfDNA samples using 10 µL of Phusion High-Fidelity PCR Master Mix (NEB), 500 nM of each primer, and 4 µL plasma cfDNA, in a final reaction volume of 20 µL. Cycling conditions were: 98 °C for 1 min, followed by 42 cycles of 98 °C for 10 s, 64 °C for 30 s, and 72 °C for 30 s, followed by 72 °C for 10 min. All amplicons for all cases are pooled into a single pool, and individual samples are tagged with Illumina indexes and purified using a MinElute PCR Purification Kit (Qiagen), quantified using a Qubit dsDNA BR Assay Kit (Invitrogen), and amplicon quality was assessed using a Bioanalyzer (Agilent). Purified PCR products were diluted to 2 nM in Elution Buffer (Qiagen), and equal amounts of all samples were pooled to give a single 2-nM library, which was denatured using sodium hydroxide according to MiSeq sample preparation instructions (Illumina) and diluted to a final concentration of 8 pM and mixed with an 8-pM PhiX control to give a 20% PhiX spike, providing sequence diversity. The library was loaded onto the Illumina MiSeq for initiation of cluster generation and a single-end 100 cycle sequencing protocol.

Confirmation of the presence of cfDNA

Where there was sufficient DNA available, the presence of cfDNA in the sample was confirmed using assays for *SRY*, *ZFY*, or *RASSAF1A*.^{10,23} Quantification of cfDNA was not undertaken.

Statistical analysis

Sensitivities and specificities with 95% confidence intervals for both approaches were calculated and 95% confidence intervals for the difference between detection rates calculated using the exact method.

RESULTS

A total of 108 cases were ascertained. Of these, 68 were referred because of a risk of achondroplasia, 53 of the 68 after 20 weeks' gestation (mean 29, range of 20–37 weeks) because of ultrasound

Table 1 *FGFR3* details of the mutations included in the next-generation sequencing panel

<i>FGFR3</i> exon	Mutation	Amino acid change	Associated skeletal disorder
6	c.742C>T	p.Arg248Cys	TD I
6	c.746C>G	p.Ser249Cys	TD I
6	c.749C>G	p.Pro250Arg	Muenke syndrome ¹⁹
6	c.749C>T	p.Pro250Leu	Craniosynostosis ²⁰
8	c.1108G>T	p.Gly370Cys	TD I
8	c.1111A>T	p.Ser371Cys	TD I
8	c.1118A>G	p.Tyr373Cys	TD I
8	c.1123G>T	p.Gly375Cys	Achondroplasia
8	c.1130T>G	p.Leu377Arg	Achondroplasia
8	c.1138G>C	p.Gly380Arg	Achondroplasia
8	c.1138G>A	p.Gly380Arg	Achondroplasia
8	c.1142T>A	p.Val381Glu	Hypochondroplasia
11	c.1619A>C	p.Asn540Thr	Hypochondroplasia
11	c.1619A>G	p.Asn540Ser	Hypochondroplasia
11	c.1620C>G	p.Asn540Lys	Hypochondroplasia
11	c.1620C>A	p.Asn540Lys	Hypochondroplasia
13	c.1948A>C	p.Lys650Gln	Hypochondroplasia
13	c.1948A>G	p.Lys650Glu	TD II
13	c.1949A>T	p.Lys650Met	TD I/SADDAN ²¹
13	c.1949A>C	p.Lys650Thr	Familial acanthosis ²² nigricans/hypochondroplasia + acanthosis nigricans
13	c.1950G>C	p.Lys650Asn	Hypochondroplasia
13	c.1950G>T	p.Lys650Asn	Hypochondroplasia
17	c.2419T>G	p.*807Glyext*102	TD I
17	c.2419T>A	p.*807Argext*102	TD I
17	c.2420G>C	p.*807Serext*102	TD I
17	c.2420G>T	p.*807Leuext*102	TD I
17	c.2421A>T	p.*807Cysext*102	TD I
17	c.2421A>C	p.*807Cysext*102	TD I
17	c.2421A>G	p.*807Trpext*102	TD I

TD I, thanatophoric dysplasia type I; TD II, thanatophoric dysplasia type II; *FGFR3*, fibroblast growth factor receptor 3.

The panel covers mutations causing achondroplasia,^{7–9,18} thanatophoric dysplasia,^{11,13,14} hypochondroplasia,^{18,19} and other rarer dysplasias^{19–22}.

findings suggestive of a skeletal problem. A further 13 were referred between 9 and 14 weeks (mean 10.3 weeks) because a previous affected pregnancy conferred a small recurrence risk due to germline mosaicism, that is, a mutation present in some germline cells but not in somatic cells, so the individual parent shows no signs of the condition but is at a very small increased risk of having an affected child. The remaining two cases were referred because of raised paternal age or because the father was affected. There were 40 cases at risk of TD, nine with a germline mosaicism recurrence risk tested between 9 and 16 (mean 12.7) weeks' gestation and the other 31 with ultrasound findings detected between 12 and 36 (mean 18.6) weeks' gestation. Three cases have been lost to follow-up, and in 11 the pregnancies are ongoing. These have all been excluded from further analysis and will not be discussed further. Not all cases were tested using both approaches, leaving 72 cases tested with PCR-RED and 47 with the NGS panel (Table 2). Twenty-seven

cases, where there was sufficient DNA or plasma available, were tested using both platforms.

An example of the results obtained in the PCR-RED assay is shown in Figure 1. The sensitivity and specificity (with 95% confidence intervals) of the PCR-RED assay if taking inconclusive results as negative were 88.6% (71–96%) and 100% (92–100%), while for the NGS panel, the sensitivity was 96.2% (81–99.3%) and the specificity, 100% (85–100%) (Table 2). Comparison of the sensitivities for each approach, as well as the comparison of the specificities, showed no significance [–7.7 (–25.4–9.1) and 0, respectively], but as the confidence intervals are wide, differences that may be of clinical significance cannot be excluded. Furthermore, the NGS assay yielded no inconclusive results and indeed gave a result in both cases where PCR-RED was inconclusive. Overall, PCR-RED was inconclusive in five cases (7%), all tested after 20 weeks' gestation with one being a twin pregnancy. In one case, testing

Table 2 The performance of the PCR-RED and NGS panel for the detection of *FGFR3* mutations causing achondroplasia and thanatophoric dysplasia with confirmed postnatal diagnoses

Confirmed postnatal diagnosis	Mutation	Prenatal test result					
		PCR-RED			NGS panel		
		True positive	False negative	Inconclusive	True positive	False negative	Inconclusive
Achondroplasia	c.1138G>A (p.Gly380Arg)	14	0	1	8	0	0
	c.835A>T (p.Ser279Cys)	ND	ND	ND	0	1 ^a	0
Thanatophoric dysplasia	c.742C>CT (p.Arg248Cys)	6 ^b	1 ^c	1 ^b	9 ^b	0	0
	c.1948A>AT (p.Lys650Glu)	3	0	0	3	0	0
	c.1118A>G (p.Tyr373Cys)	ND	ND	ND	2	0	0
	c.746C>G (p.Ser249Cys)	ND	ND	ND	2	0	0
	c.2419T>A (p.*807Argext*101)	ND	ND	ND	1	0	0
^d Does not carry <i>FGFR3</i> mutation	No mutant allele detected	43 ^b	0	3	20 ^b	0	0
	Total	66	1	5	46	1	0
	Sensitivity	88.6% (95% CI 71–96) taking inconclusive that were positive as false negative				96.2% (81–99)	
	Specificity	100% (92–100)				100% (85–100)	
	Positive predictive value	100% (86–100)				100% (87–100)	
	Negative predictive value	93.9 (83.5–97.9) taking inconclusive that were positive as false negative				95.5% (78.2–99.2)	
	Inconclusive rate	7%				0	

ND, not done; PCR-RED, PCR-restriction enzyme digest; NGS, next-generation sequencing; *FGFR3*, fibroblast growth factor receptor 3.

^aFalse negative for rare achondroplasia mutation c.835A>T (p.Ser279Cys). Panel redesigned to incorporate this mutation.

^bIncludes one twin pregnancy, one twin normal, and one with a skeletal dysplasia. The case that was inconclusive using PCR-RED for c.742C>CT (p.Arg248Cys) was positive when tested with NGS.

^cFalse negative for c.742C>CT (p.Arg248Cys) due to low fetal fraction. There was insufficient sample available to analyze this sample using NGS.

^dIncludes normal, growth retarded fetuses and other skeletal dysplasias.

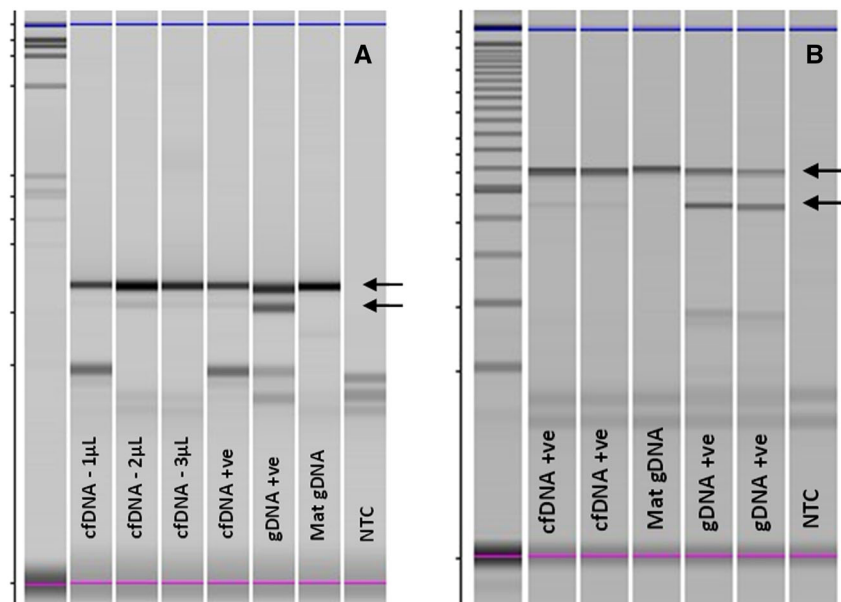


Figure 1 PCR-restriction enzyme digest results for (a) achondroplasia [fibroblast growth factor receptor 3 (*FGFR3*): c.1138G>A p. (Gly380Arg)/restriction enzyme BsrG1] and (b) thanatophoric dysplasia [*FGFR3*: c.742C>T p.(Arg248Cys)/restriction enzyme DraIII]. Upper arrows indicate wild-type (normal) allele that is strongly present in all samples. Bottom arrows indicate the mutant alleles that are faint in affected cell-free DNA (cfDNA+) and stronger mutation positive control genomic DNA (gDNA+ve). There is no mutant band present in the unaffected maternal genomic DNA (Mat gDNA). The figure shows uncropped images visualized on Shimadzu Multi-NA microchip electrophoresis system. NTC, blank, no template control

for an independent fetal marker was also inconclusive, and, as the sample was very small, repeat testing could not be performed. In two further cases, *SRY* assays confirmed the presence of cfDNA, but the *FGFR3* PCR-RED assay was inconclusive. In the last two, including the twin pregnancy, there was sufficient sample to run the NGS panel as well, and this detected the wild-type or mutant *FGFR3* allele in both cases. Both the PCR-RED and NGS assays have high positive predictive values of 100% (86–100%) and 100% (87–100%), respectively, with negative predictive values of 93.9 (83.5–97.9%) and 95.5 (78.2–99.2%), respectively (Table 2). There was one false negative result using PCR-RED where a c.742C>CT (p.Arg248Cys) TD-causing mutation was not detected at 21 weeks' gestation although the *RASSAF1A* assay had confirmed the presence of cfDNA. With NGS, one case was reported as 'no mutation detected' but postnatally found to be heterozygous for a very rare achondroplasia-causing mutation [c.835A>T (p.Ser279Cys)],¹⁸ which was not included in the panel at that time. The panel has since been modified to cover this mutation.

DISCUSSION

This study represents the largest reported data set for the NIPD of *de novo* or paternally inherited autosomal dominant monogenic disorders and confirms that analysis of cfDNA in maternal plasma offers a safe alternative approach to invasive prenatal diagnosis. These data clearly demonstrate that NGS provides a more sensitive approach than PCR-RED, as it detected mutations in cases that were reported as inconclusive using a PCR-RED assay while yielding no inconclusive results. While PCR-RED is a very cost-effective technique, which is widely used in laboratories that do not have access to NGS platforms, mutations have to be screened for individually, interpretation of agarose gel electrophoresis can be very subjective (Figure 1a and b), and it is only possible to test for mutations where a restriction enzyme cleavage site is created or destroyed by the mutation and therefore cannot be applied to all mutations. Thus, while this approach may be of use when testing for the recurrence of a known mutation for which an assay can be designed, when attempting diagnosis in cases presenting *de novo*, it would be very time consuming to use PCR-RED to individually test for all possible disease-causing mutations.

To date, there has been relatively little NIPD available clinically for families at high risk of single gene disorders, with most reported cases being performed on a research basis.⁹ Fetal sex determination in pregnancies at risk of sex-linked disorders to enable targeting of invasive testing in male-bearing pregnancies is in use in many parts of Europe and has been the standard of care in the UK NHS since 2011 (<http://ukgtn.nhs.uk/news-events/article/non-invasive-prenatal-diagnosis-nipd-ukgtn-approval-of-gene-dossiers-for-fetal-sex-determination-for-congenital-adrenal-hyperplasia-cah-and-x-linked-conditions-excluding-hemophilia-7/>).²⁴ This non-invasive approach is welcomed by women and health professionals alike,²⁵ as it avoids the risk of miscarriage and can be carried out earlier in pregnancy. The PCR-RED approach to NIPD for achondroplasia and TD was approved for use in the UK NHS in 2012. Although there are relatively few reports of NIPD for single

gene disorders, a recent survey of women who had undergone this testing reported that they too valued the safety, ease of access, and early testing available.²⁶ Health professionals, who have also been found to view the introduction of NIPD for single gene disorders positively, however, emphasized the need for new tests to be highly accurate and thoroughly validated.²⁷

Non-invasive prenatal diagnosis can be a very useful aid to clinical management. TD is a condition that is increasingly detected by ultrasound early in pregnancy,¹² but the differential diagnosis includes the short-ribbed polydactyly syndromes and other autosomal recessively inherited conditions associated with a high recurrence risk, while TD is a new dominant mutation with a low recurrence risk. If positive for TD, NIPD can allow for a definitive diagnosis without recourse to invasive testing and allows the option of a surgical termination, as a post-mortem will not be required. NIPD can also significantly alter clinical management in multiple pregnancies where the fetuses are discordant for abnormalities, as in three of the cases reported here. In this situation, definitive diagnosis using NIPD avoids the risk of miscarriage for the normal fetus. In addition, in lethal conditions, such as TD, it allows for conservative management of pregnancy with no requirement for feticide. In cases presenting late in pregnancy at risk of achondroplasia, NIPD allows for definitive diagnosis and accurate parental counseling without risk of precipitating preterm labor. In all cases, it also allows for a safe, non-invasive test early in future pregnancies to exclude a recurrence or inheritance of a paternal mutant allele. This can be carried out from 9 weeks' gestation, earlier than invasive testing, which cannot safely be performed until 11 weeks¹ and much earlier than an ultrasound scan for those women not wanting to put the pregnancy at risk with an invasive test. Early access to NIPD in these circumstances has been welcomed by parents and has improved access to prenatal diagnosis for families where moral or religious reasons necessitate testing very early in pregnancy.^{25,26}

Overall, our data demonstrate that NGS will be the best approach for NIPD for many monogenic conditions. Unlike PCR-RED, it is possible to screen for all mutations in a single assay, making it appropriate both for the exclusion of a recurrence and for diagnosis in cases arising *de novo*. The digital output from NGS is easy to interpret although in view of the low level of background counts in normal samples, maternal genomic DNA should be analyzed alongside the cfDNA sample (Table 3). NGS offers optimal turnaround times as the entire procedure can be carried out in 24 h, which allows timely reporting for a prenatal test result. Furthermore, multiple patients with different conditions can be analyzed in a single run, allowing optimization of the workflow in a routine NHS diagnostic laboratory. Finally, the use of NGS may allow definitive NIPD in autosomal recessive conditions or X-linked where there is a high background of mutant allele in maternal plasma as she is a carrier herself. In this situation, NIPD cannot depend on detecting the presence or absence of an allele not present in the mother but requires an assessment of the relative proportion of mutant allele because, if the fetus is affected, there will be more mutant than wild-type allele

Table 3 Mutation counts

Target Sequence	ACH Case 9		ACH Case 10		TD Case 17	TD Case 21		TD Case 22		TD Case 23	
	cfDNA	Mat gDNA	cfDNA	Mat gDNA	cfDNA	cfDNA	Mat gDNA	cfDNA	Mat gDNA	cfDNA	Mat gDNA
Exon 6 – wt					21 386	7046	24 897	11 975	24 071	65 527	90 155
c.742C>T					<u>703</u>	<u>1</u>	7	1	2	12	18
c.749C>G					0	<u>180</u>	2	2	<u>2</u>	4	1
c.749C>G					2	0	5	3	7	2	3
c.749C>T					1	3	8	2	4	14	18
Exon 8 – wt 1	59 065	163 007	103 269	48 748	44 888					223 530	228 583
c.1108G>T	10	18	9	6	1					30	27
c.1111A>T	18	41	39	23	15					33	35
c.1118A>G	8	22	16	5	13					<u>8487</u>	<u>26</u>
Exon 8 – wt 2	5183	13 112	3794	1737	12 584					6999	5753
c.1123G>T	15	43	13	3	8					9	3
c.1130T>G	12	35	7	4	2					98	99
c.1138G>C	60	95	39	16	66					10	4
c.1138G>A	<u>515</u>	<u>19</u>	<u>207</u>	<u>0</u>	11					1	4
Exon 8 – wt 3	114	297	178	97	208					119	159
c.1142T>A	2	16	5	9	3					21	38
Exon 11 – wt					0	43 894	94 651	56 888	63 064	166 808	235 016
c.1619A>C					0	10	37	15	14	27	47
c.1619A>G					0	4	2	5	6	20	23
c.1620C>G					0	1	0	2	1	6	9
c.1620C>A					0	10	24	16	17	40	53
Exon 13 – wt					111 829	84 573	127 642	70 368	91 694	264 715	247 971
c.1948A>C					14	7	15	11	6	29	31
c.1948A>G					104	9	19	<u>4139</u>	<u>2</u>	39	29
c.1949A>T					10	4	9	4	9	36	41
c.1949A>C					62	25	43	18	28	39	60
c.1950G>C					5	2	6	6	0	21	10
c.1950G>T					82	21	46	28	32	52	42
Exon 17 – wt					35 976	37 747	105 463	48 311	53 519	121 602	229 702
c.2419T>G					287	67	278	103	133	858	1662
c.2419T>A					6	12	30	8	11	15	22
c.2420G>T					15	41	93	52	53	46	94
c.2420G>C					3	1	8	3	3	17	18
c.2421A>T					0	6	5	5	1	12	22
c.2421A>C					20	32	105	55	61	25	62
c.2421A>G					10	3	13	7	7	15	41

ACH, achondroplasia; TD, thanatophoric dysplasia; wt, wild type or non-diseased causing; cfDNA, cell-free DNA; Mat gDNA, maternal genomic DNA.

Case 17 was analyzed early in the NGS series, before we routinely ran the maternal gDNA in parallel to facilitate interpretation in view of the low background counts in normal samples. Disease-causing mutations are shown in bold, highlighted, and underlined. Note the difference between fetal and maternal counts.

present in maternal plasma. However, the amount of cfDNA (the fetal fraction) in maternal plasma varies between and within women and will directly affect the total amount of mutant allele in plasma if the fetus is affected. Estimation of the fetal fraction requires measurement of a fetal DNA marker

present in the fetus but not in the mother.²⁸ While this is possible in male-bearing pregnancies using *SRY* or *DYS14* alleles, there is no universal marker for female fetuses. If using the NGS approach, panels of single nucleotide polymorphisms with high degrees of heterogeneity can be incorporated into

the panel to facilitate estimation of fetal fraction. Furthermore, NGS offers the potential for a haplotyping approach using single nucleotide polymorphisms closely linked to the disease-causing gene in conditions where the mutations are not amenable to direct detection.²⁹

CONCLUSION

The development of cfDNA aneuploidy testing has been driven by the commercial sector,^{3–5} as there is a large market opportunity. Implementation of NIPD for monogenic disorders has not attracted such attention, largely because development must be on a patient or disease-specific basis and demand is less. Here, we show how NGS can offer a comprehensive and speedy approach to NIPD for *de novo* and paternally inherited autosomal dominant conditions, which is welcomed by patients and health professionals alike and which can significantly improve clinical management. With increasing availability of sequencing platforms in public

sector laboratories, it is hoped that there will be developments in NIPD for a wider range of monogenic disorders for the benefit of these families who are at high risk of genetic disease.

WHAT'S ALREADY KNOWN ABOUT THIS TOPIC?

- Non-invasive prenatal diagnosis (NIPD) using PCR-based methods has been reported for the detection or exclusion of individual paternally inherited or *de novo* alleles in maternal plasma.

WHAT DOES THIS STUDY ADD?

- NIPD using next generation sequencing provides an accurate, more sensitive approach which can be used to detect multiple mutations in a single assay and so is ideal when screening a gene with multiple potential pathogenic mutations. Next generation sequencing thus provides a flexible approach to non-invasive prenatal diagnosis ideal for use in a busy service laboratory.

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