The role of sonographic phenotyping in delivering an efficient non-invasive prenatal diagnosis (NIPD) service for *FGFR3*-related skeletal dysplasias

Running title	Sonographic phenotyping in NIPD for FGFR3-related skeletal dysplasias
Word count	3316
Tables	3
Figures	1
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Funding	RM is a clinical training fellow fully funded by the NIHR Biomedical Research Centre at Great Ormond Street Hospital. LSC is an NIHR Senior Investigator and is partially funded by the NIHR Biomedical Research Centre at Great Ormond Street Hospital. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health
Conflict of interest	None
Data availability statement	Data sharing is not applicable to this article as this study relates to a clinical service audit. The raw data that support the findings of this study are not publicly available due to privacy or ethical restrictions.

What is already known about this topic?

• The sonographic features of achondroplasia and thanatophoric dysplasia are well documented but these conditions can still be difficult to diagnose and so molecular confirmation is required for definitive diagnosis.

• Non-invasive prenatal diagnosis (NIPD) based on analysis of cell free DNA in maternal plasma offers safe, accurate molecular diagnosis of *FGFR3*-related skeletal dysplasias.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/pd.5687

What does this study add?

• The diagnostic yield of NIPD for *FGFR3* mutations is maximised by accurate sonographic phenotyping, with yields over 80% achieved when several characteristic sonographic features of achondroplasia or thanatophoric dysplasia are present.

Abstract

Objectives:
To evaluate the diagnostic yield of non-invasive prenatal diagnosis (NIPD) for *FGFR3*-related skeletal dysplasias and assess the accuracy of referrals based on sonographic findings to inform guidelines for referral.

Methods:
We retrospectively reviewed laboratory and referral records from 2012-2018 to ascertain all NIPD tests performed using our next generation sequencing panel to detect *FGFR3* mutations. We calculated the diagnostic yield of the test overall and when sub-divided according to the phenotypic features identified on ultrasound before testing. Pregnancy outcomes were ascertained wherever possible from referring centres.

Results:
Of 335 tests, 261 were referred because of sonographic findings, of which 80 (31.3%) had a mutation. The diagnostic yield when short limbs were the only abnormal sonographic feature reported was 17.9% (30/168), increasing to 48.9% (23/47) in the presence of one, and 82.6% (19/23) in the presence of two or more characteristic features in addition to short limbs.

Conclusions:
Accurate sonographic phenotyping can maximise the diagnostic yield of NIPD in fetuses suspected to have *FGFR3*-related skeletal dysplasias. We suggest that clear guidelines for referral are necessary to increase benefits, decrease costs by preventing unnecessary NIPD, and potentially allow first-line broader spectrum testing for fetuses where the aetiology may be more heterogeneous.

Keywords

FETAL CELLS, NUCLEIC ACIDS & PROTEINS, SINGLE GENE DISORDERS, Fetal ultrasound, NIPD, Skeletal dysplasia, FGFR3

The role of sonographic phenotyping in delivering an efficient non-invasive prenatal diagnosis (NIPD) service for *FGFR3*-related skeletal dysplasias

Introduction

Skeletal dysplasias are a genetically and phenotypically heterogenous group of disorders, which can be difficult to accurately diagnose sonographically before birth due to phenotypic overlap between multiple different conditions.[1] Achondroplasia is the most common non-lethal skeletal dysplasia, with an incidence of 3-6 per 100 000 livebirths.[2] It is caused by pathogenic variants in the *FGFR3* gene; in ~98% of cases this is a c.1138G>A missense mutation and in a further 1% is a c.1138G>C mutation.[3,4] Inheritance is autosomal dominant but the majority (~80%) of cases arise sporadically due to a *de novo* mutation of paternal origin.[5] The cardinal features of achondroplasia are rhizomelic limb shortening occurring after 25 weeks gestation and relative macrocephaly with frontal bossing. Other features such as brachydactyly and 'trident hand' are also commonly observed, with mild femoral bowing and a small chest reported less frequently. Polyhydramnios is a common feature in the late third trimester.[6]

Thanatophoric dysplasia (TD) is the most common lethal skeletal dysplasia, with an incidence of 2-3 per 100 000 births.[2] It is characterised by severe limb shortening, macrocephaly, frontal bossing, trident fingers and a narrow thorax that results in pulmonary hypoplasia and respiratory failure. Two different forms are recognised, identifiable by different radiological features in addition to the short limbs and small chest: TD Type I is distinguished by curved femurs, platyspondyly and infrequent craniosynostosis, whereas TD Type II has straight femurs and a 'cloverleaf' skull. Thanatophoric dysplasia also results from *de novo* pathogenic variants in *FGFR3* but, in contrast to achondroplasia, there are multiple known disease causing variants. Type I is caused by a c.742C>T mutation in over 50% of cases and multiple other mutations are also reported.[7] In TD Type II, a single c.1948A>G mutation is responsible for all reported cases[7] Unlike achondroplasia, TD presents in early pregnancy, with short limbs detectable on fetal ultrasound from as early as 13 weeks' gestation.[8]

Molecular diagnosis is required for definitive diagnosis due to the commonality of several features between these and other skeletal dysplasias. This is desirable for accurate counselling and pregnancy management as the prognosis and outcomes vary considerably between conditions. Further, an important differential diagnosis for fetuses presenting with short limbs in the second or early third trimester is intrauterine growth restriction (IUGR), which again has different counselling, prognostic and management implications.

The development of non-invasive prenatal diagnosis (NIPD) based on analysis of cell free DNA (cfDNA) in maternal plasma offers safer, accurate definitive diagnosis of monogenic disorders.[9] Since 2012 our UK National Health Service (NHS) laboratory has delivered an NIPD service, available from nine weeks' gestation, for *FGFR3*-related skeletal dysplasias using a Next Generation Sequencing (NGS) panel approach to detect a range of *FGFR3* mutations.[10] Safe access to definitive diagnosis has increased uptake for prenatal testing and it is important to ensure this testing is directed appropriately to deliver an efficient service and maximise benefits to patients. Accurate referral enables rapid targeted molecular confirmation and published clinical decision aids and fetal size charts exist to assist referring clinicians.[6,8,11]

Here we review our NIPD service for *FGFR3* mutations to evaluate the diagnostic yield of NIPD for *FGFR3*-related skeletal dysplasias, assess the accuracy of referrals based on sonographic findings, explore outcomes after testing and determine if further guidelines for referral are required.

Methods

NIPD Service

Our accredited Regional Genetics Laboratory delivers NIPD for *FGFR3*-related skeletal dysplasias, which include achondroplasia and TD, using an NGS approach to analyse cfDNA in maternal plasma for a panel of 29 known disease-causing mutations in the *FGFR3* gene. This service has been offered since July 2012, accepting referrals from across the UK and internationally. Pregnancies at risk are identified and referred for testing following either suggestive sonographic findings or a relevant family history in a previous pregnancy or a parent. Our current testing criteria guidelines, available at https://ukgtn.nhs.uk/uploads/tx_ukgtn/FGFR3_Related_Skeletal_Dysplasia_Panel_Test_NIPD_TC_Sept_13_renamed_July17.pdf are summarised in Figure 1. If referring clinicians wish to request testing

outside of these criteria it is recommended that they discuss with our laboratory first. Results are typically issued within five days of sample receipt.

Case ascertainment

We received local approval (audit registration number 1925) to evaluate the NIPD service delivered to date. Laboratory records spanning 6 years, from the start of the service in July 2012 until July 2018, were searched to ascertain all NIPD performed for pregnancies at risk of *FGFR3*-related skeletal dysplasias because of ultrasound findings or relevant family history. Where a test failed initially, for example due to low fetal fraction or sequencing failure, but was repeated successfully on a further maternal blood sample in the same pregnancy (10 cases) this was counted as a single test. Where a test failed and could not be repeated as it was not technically possible or no further sample was provided (4 cases) this was counted but excluded from analysis of diagnostic yields. Also excluded from the analysis of diagnostic yields were one false positive result and one negative result with a final diagnosis of achondroplasia caused by a very rare variant not included on the *FGFR3* gene panel at that time. These cases are detailed below. The indication for referral (as recorded on the test request form) in each case was examined, including review of attached ultrasound reports where available. Test results were reviewed to determine the diagnostic yield of NIPD overall and when sub-divided by sonographic features reported (Table 1), and gestational age at detection (Table 2). Pregnancy outcomes were ascertained wherever possible from referring centres, including postnatal confirmation of the prenatal molecular diagnosis by molecular testing, radiology or pathology where performed (Table 3).

Results

During the period from July 2012-July 2018, a total of 335 NIPD tests were performed for *FGFR3* mutations. Of these, 4 (1.2%) were done because the father was affected with achondroplasia, 67 (20%) were because of a previous affected pregnancy, and 261 (77.9%) following detection of sonographic abnormalities suggestive of achondroplasia or thanatophoric dysplasia. Three cases were referred privately for NIPD for other reasons outside of our usual guideline: one for increased

risk of achondroplasia in light of advanced paternal age, and two at parental request for reassurance where there was a history of members of the wider family affected by achondroplasia. In total, 80 diagnoses were made: 40 of achondroplasia, 36 of TD and 4 of hypochondroplasia. Two of the diagnoses of achondroplasia were made in pregnancies at known 50% risk due to an affected father, and all other diagnoses were made in pregnancies referred due to sonographic findings. There were no positive results amongst the 67 pregnancies at very low risk of recurrence due to possible germline mosaicism.

In the first year of the service there was one negative result where sonographic features were not entirely typical for either achondroplasia or TD but a diagnosis of achondroplasia was identified after birth, due to a very rare pathogenic variant (c.835A>T) that was not included on the panel at the time. This is a recognised limitation of a panel-based test and the variant was subsequently added to the panel. Another case, with sonographic abnormalities, was a false positive for achondroplasia (c.1138G>A) but was eventually found to have a *COL2A1*-related skeletal dysplasia after birth. This false positive occurred due to poor amplification of cfDNA and a false variant call resulting from the formation of primer dimers during the PCR stage of library preparation for the assay. More stringent quality control checks were subsequently implemented, which this sample would not have passed. Additionally, four tests (1.2%) failed or were inconclusive and no diagnosis could be made: three due to samples failing quality control checks and one due to low fetal fraction. Of the inconclusive results, one was in a test requested because of a family history of achondroplasia and the other three were in tests done because of sonographic findings.

Diagnostic yield based on phenotypic features

Of the 261 tests done because of sonographic findings, five were excluded from further analysis due to failed/inconclusive/incorrect results, as described above. A pathogenic variant was detected in 80/256 successful tests, giving an overall diagnostic yield of 31.3%. This comprises a diagnostic yield of 25.7% (44/171) for achondroplasia/hypochondroplasia and a diagnostic yield of 42.4% (36/85) for TD (Table 1).

The total diagnostic yield for fetuses referred with short limbs only was 16.6% (27/163), increasing to 49% (24/49) in the presence of one, and 80.8% (21/26) in the presence of two or more characteristic features in addition to short limbs. For achondroplasia specifically, the presence of

either relative macrocephaly or frontal bossing in addition to short limbs raised the diagnostic yield dramatically (from ~16% to >70%). For TD specifically, the presence of a small chest/short ribs in addition to short limbs increased the diagnostic yield from 16.2% to 57.9%. Another characteristic feature of both conditions, short fingers, was infrequently commented upon in the referrals received but where reported, 5/7 (71.4%) fetuses with short limbs and short fingers had achondroplasia or TD.

Diagnostic yield for achondroplasia based on gestational age

For pregnancies suspected at risk of achondroplasia based on sonographic findings, 39 referrals for NIPD were made before 25 weeks of gestation, and 132 referrals after 25 weeks. In pregnancies presenting after 25 weeks of gestation, a diagnosis was made in 43/132 (32.6%) cases, compared to only one diagnosis (2.6%) from 39 cases presenting at an earlier gestation (Table 2). This was a diagnosis of hypochondroplasia in a fetus presenting with short long bones only, at 22 weeks of gestation. Furthermore, three cases presenting before 25 weeks of gestation were initially referred with a suspicion of achondroplasia but NIPD diagnosed TD. These findings are consistent with the natural history of achondroplasia and highlight that short limbs apparent at earlier gestations are more likely to have a different aetiology.

Maternal and fetal doppler findings

Information on maternal and fetal arterial doppler measurements was provided in 44 (of 171) cases referred for testing after detection of sonographic abnormalities suggestive of achondroplasia. Where this information was available, 10/40 pregnancies with normal dopplers, thereby making IUGR unlikely, had a pathogenic *FGFR3* variant detected. None of the four pregnancies with abnormal dopplers had a pathogenic variant detected, and all resulted in normal live births.

Pregnancy outcomes

Information regarding pregnancy outcome was available for 154/335 tests done during this period. Where outcome information was available, all cases of prenatally diagnosed *FGFR3*-related skeletal dysplasia were confirmed clinically or molecularly at birth or post-mortem (excluding the one false positive result described above).

Where no *FGFR3* mutation was detected and pregnancy outcome data was available, the most frequent outcome was healthy live birth (accounting for 25/26 cases where the indication for testing was recurrence risk or known familial risk and 44/79 cases where testing was done due to sonographic abnormalities). In those pregnancies tested due to abnormal ultrasound findings, an alternative explanation was found postnatally or post-mortem in 34/79 cases; these are listed in Table 3 with additional information on sonographic findings available in Supplementary Table 1. One pregnancy ended in termination with no post-mortem examination and remained undiagnosed.

Discussion

Test uptake

Here we have demonstrated the sustained successful use of a NGS panel approach to deliver fast and accurate NIPD for *FGFR3*-related skeletal dysplasias in a busy public health service diagnostic laboratory. We have seen uptake of *FGFR3* testing in our NIPD service rise steadily since its introduction, from 26 tests performed between July 2012-July 2013, to 74 tests between July 2017-July 2018. Our experience of high uptake is consistent with findings in the literature of overwhelmingly positive attitudes towards NIPD for single gene disorders from parents, who value the test's safety and speed of results.[12–14] Of note, as well as pregnancies with ultrasound abnormalities and those at known 50% risk of achondroplasia due to an affected parent, we have also seen high uptake of NIPD for *FGFR3* mutations (20% of tests done) amongst unaffected couples with a previous affected pregnancy and a very low recurrence risk due to the possibility of germline mosaicism. Some of these families request NIPD where they would previously have declined invasive testing due to the small risk of miscarriage. To date, we have not encountered any instances of recurrence due to germline mosaicism, and some may question whether testing for this indication represents a cost-effective use of resources.[15] This raises wider ethical issues regarding eligibility in a public healthcare setting for testing in families at very low risk of recurrence. However, we must not undervalue the emotional and psychological benefits families derive from early reassurance and 'normalisation' of the pregnancy, without risking the loss of a healthy pregnancy through invasive testing.[12]

Diagnostic yield and accuracy of referrals

For pregnancies referred due to sonographic abnormalities our NIPD service has achieved an overall diagnostic yield of 31.3% for *FGFR3*-related skeletal dysplasia. As well as diagnosing achondroplasia and TD, we have also identified four cases of hypochondroplasia, highlighting the value of using a panel-based approach over testing for common variants only. Of the test-negative cases where follow up information was available around one third were affected by other skeletal dysplasias, genetic disorders or undiagnosed dysmorphic syndromes. Review of the sonographic findings in these cases (Supplementary Table 1) reveals some overlap with features of *FGFR3*-related skeletal dysplasias but these fetuses tended to lack the more specific features, or had additional features not typical of achondroplasia or TD. The remaining test-negative cases with follow-up information available had no structural abnormalities detected postnatally. Some of these infants were however noted to be growth-restricted or of low birthweight. It is notable that none of the infants with a documented normal outcome or IUGR diagnosis presented with any sonographic features other than 'short limbs', suggesting that the presence of short limbs in isolation may not be a strong indication for NIPD targeted at *FGFR3* mutations.

This was further explored in our analysis of diagnostic yield by number of phenotypic features, where we observed that pathogenic *FGFR3* variants were detected in just 16.6% of cases referred with short limbs only, but saw a dramatic increase in diagnostic yields when one or more specific features of achondroplasia or TD were noted on ultrasound at the time of referral. In particular, the presence of macrocephaly or frontal bossing substantially increased the likelihood of detecting an achondroplasia-causing *FGFR3* mutation, and the presence of a small chest increased the likelihood of detecting a TD-causing mutation. This underlines how the accuracy of referrals influences test outcome, and the critical importance of high quality phenotyping at the point of referral. Phenotyping in the fetus can be notoriously difficult as examination is limited by the resolution of ultrasound scanning, which may be compromised in the third trimester when achondroplasia typically presents, and requires a skilled operator. However we suggest that better adherence to our guidelines for referral could increase diagnostic yields and reduce unnecessary tests.

Referring clinicians must also bear in mind the natural history of the suspected condition. For example, achondroplasia is an unlikely aetiology for short limbs in a fetus presenting before the third

trimester, as limb growth in achondroplasia is generally preserved at earlier gestations. This is reflected in our finding that a pathogenic variant (causing hypochondroplasia) was detected in only one pregnancy referred for achondroplasia testing before 25 weeks' gestation.

Long bone shortening is also a relatively common presentation of IUGR secondary to placental insufficiency.[16,17] This is why we recommend performing maternal and fetal doppler examination as a marker of placental insufficiency, as well as reviewing past obstetric history and the results of any Down syndrome screening test results before referring for NIPD for achondroplasia. In our cohort, doppler measurements were infrequently included in the referrals we received but some pregnancies tested did go on to receive a diagnosis of IUGR. Therefore, for short-limbed fetuses presenting in the third trimester, we suggest that consistent recording of doppler measurements within the referral information would also help to guide genetic testing.

Towards improved guidelines for testing

NIPD for *FGFR3*-related skeletal dysplasias, along with NIPD for other monogenic disorders and broader prenatal genomic testing, will soon become more widely available in clinical practice within the UK NHS Genomic Medicine Service and worldwide. As testing options for prenatal diagnosis increase and we move increasingly towards embedding genomic testing into mainstream clinical care it is essential to disseminate clear guidelines to aid clinicians in selecting the most appropriate testing strategy for patients. For prenatal diagnosis this is crucial to ensure that a diagnosis is reached quickly, to allow families the greatest choice of management options and as much time as possible for decision-making. Further, in a resource-constrained public healthcare system it is important to choose the right testing for each patient to ensure equitable and efficient use of resources.

As discussed above, accurate phenotyping at the point of referral is key to targeting prenatal genetic testing appropriately. Where achondroplasia or TD is suspected clinically NIPD offers safe, accurate and rapid molecular confirmation. On the other hand, where fetal sonographic features are non-specific or the differential diagnosis is broad then it may be more appropriate to choose a broader first-line investigation. For example, rapid fetal exome sequencing from amniocytes or chorionic villi will soon be available within the UK NHS Genomic Medicine Service for pregnancies complicated by unexpected fetal structural abnormalities with a likely monogenic aetiology. This will enable the

assessment of many candidate genes in a single test, facilitating faster diagnosis than sequential testing of single genes where the differential diagnosis is broad. For fetuses with a suspected skeletal dysplasia this has already been shown to have very high diagnostic rates.[18,19] At present, exome sequencing does require an invasive test in pregnancy but larger gene panels for NIPD are also likely to become available in future.

Therefore, we suggest the consistent use of guidelines for NIPD referral which highlight the combination of ultrasound findings most suggestive of achondroplasia or TD, and refinement of existing guidelines to signpost alternative testing options for those pregnancies less likely to benefit from NIPD for *FGFR3* mutations. For the diagnosis of achondroplasia, one potential strategy proposed recently by Vivanti and colleagues[11] uses a decision tree based on sonographic findings whereby NIPD is offered during the second trimester only where at least one other typical feature of achondroplasia is present in addition to short limbs. In cases of isolated limb shortening the authors recommend repeat ultrasound scanning after 26 weeks' gestation and an offer of NIPD at this time if the limb shortening has worsened or any additional features of achondroplasia have developed. This group estimated that use of this staged approach would avoid an offer of NIPD in 75% of negative cases. Our findings support the use of a similar strategy since diagnostic yield for achondroplasia was 52% where any one additional sonographic feature was present compared to 16.7% for short limbs only, and only one case of hypochondroplasia was diagnosed in referrals before 25 weeks.

Since TD presents at an earlier gestation, such a staged approach to ultrasound scanning is not possible, however our findings indicate a similarly low diagnostic yield (16.2%) when pregnancies are referred for NIPD due to isolated limb shortening. Therefore we suggest offering NIPD only where at least one additional characteristic sonographic feature is present.

Conclusion

The sonographic features of achondroplasia and TD are well documented. Here we have shown how careful case selection based on these sonographic features can improve the diagnostic yield of NIPD in fetuses at risk of these conditions by allowing targeted molecular confirmation. Patients and healthcare providers welcome NIPD as it offers safer, accurate and timely prenatal diagnosis for

these skeletal dysplasias. Demand for NIPD for these conditions has been high, with families using the diagnostic information to inform pregnancy management, prepare for the birth, guide plans for safe delivery and postnatal management, and define recurrence risks for future reproductive planning. We suggest that clear guidelines for referral are necessary to decrease costs and increase benefits, preventing unnecessary NIPD and potentially allowing first-line broader spectrum testing for fetuses where the aetiology may be more heterogeneous.

Acknowledgements

This work is supported by the NIHR GOSH BRC. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

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	Achondroplasia & Hypochondroplasia		Thanatophoric dysplasia		Combined	
Referral reason	**No. of tests**	**No. mutation positive (%)**	**No. of tests**	**No. mutation positive (%)**	**No. of tests**	**No. mutation positive (%)**
Features not specified on referral	***10***	***2 (20%)***	***8***	***6 (75%)***	***18***	***8 (44.4%)***
Unspecified scan abnormalities/no clinical features listed	8	0	4	3 (75%)	12	3 (25%)
Clinician suspicion of ACH/TD	2	2 (100%)	4	3 (75%)	6	5 (83.3%)
Short limbs only	***126***	***21 (16.7%)***	***37***	***6 (16.2%)***	***163***	***27 (16.6%)***
Two features	***25***	***13 (52%)***	***24***	***11 (45.8%)***	***49***	***24 (49%)***
Short limbs + relative macrocephaly	7	5 (71.4%)	-	-	7	5 (71.4%)
Short limbs + frontal bossing	8	7 (87.5%)	-	-	8	7 (87.5%)
Short limbs + short fingers	1	0	-	-	1	0
Short limbs + polyhydramnios	3	1 (33.3%)	-	-	3	1 (33.3%)
Short limbs + small chest	3	0	19	11 (57.9%)	21	10 (47.6%)
Short limbs + bowed femora	4	0	4	0	8	0
Short limbs + cloverleaf skull	-	-	1	0	1	0
More than two features	***10***	***8 (80%)***	***16***	***13 (81.3%)***	***26***	***21 (80.8%)***
Short limbs + relative macrocephaly + short fingers	1	1 (100%)	-	-	1	1 (100%)
Short limbs + relative macrocephaly + frontal bossing	1	1 (100%)	-	-	1	1 (100%)
Short limbs + relative macrocephaly + frontal bossing + short fingers	1	1 (100%)	-	-	1	1 (100%)

Final diagnosis/outcome	Number of					
Short limbs + relative macrocephaly + small chest	2	2 (100%)	-	-	2	2 (100%)
Short limbs + frontal bossing + short fingers	1	1 (100%)	-	-	1	1 (100%)
Short limbs + frontal bossing + polyhydramnios	1	1 (100%)	-	-	1	1 (100%)
Short limbs + polyhydramnios + short fingers	1	0	-	-	1	0
Short limbs + polyhydramnios + small chest	1	1 (100%)	-	-	1	1 (100%)
Short limbs + small chest + frontal bossing	1	0	2	2(100%)	3	2 (66.7%)
Short limbs + small chest + bowed femora	-	-	7	4 (57.1%)	7	4 (57.1%)
Short limbs + small chest + cloverleaf skull	-	-	2	2 (100%)	2	2 (100%)
Short limbs + small chest + short fingers	-	-	2	2 (100%)	2	2 (100%)
Short limbs + bowed femora + frontal bossing	-	-	1	1 (100%)	1	1 (100%)
Short limbs + small chest + bowed femora + relative macrocephaly	-	-	1	1 (100%)	1	1 (100%)
Short limbs + small chest + bowed femora + relative macrocephaly + polyhydramnios	-	-	1	1 (100%)	1	1 (100%)
TOTALS	**171**	**44 (25.7%)**	**85**	**36 (42.4%)**	**256**	**80 (31.3%)**

Table 1. Diagnostic yield of NIPD for *FGFR3*-related skeletal dysplasias by sonographic features

Gestation at referral	No. of tests	No. mutation positive (%)
Before 25 weeks	39	1 (2.6%)
25 weeks or later	132	43 (32.6%)
TOTAL	**171**	**44 (25.7%)**

Table 2. Diagnostic yield of NIPD for achondroplasia/hypochondroplasia by gestation

	cases (n=80)
Live birth with no apparent abnormality detected	44
Recognised genetic skeletal dysplasia (Osteogenesis imperfecta x6, Spondyloepiphyseal dysplasia, 3M syndrome x2, Achondrogenesis type II, prenatal Caffey disease, Kniest dysplasia)	12
Unrecognised skeletal dysplasia	9
Intrauterine growth restriction (IUGR)	6
Other monogenic or chromosomal disorder (Trisomy 13, Trisomy 21, Inclusion cell disease, Spinal muscular atrophy)	4
Unrecognised dysmorphic syndrome	3
Fetal anaemia (underlying cause not apparent)	1
Termination of pregnancy with no post-mortem	1

Table 3. Pregnancy outcomes in cases referred due to abnormal sonographic findings where NIPD for *FGFR3* mutations was negative

UK Genetic Testing Network testing criteria for NIPD for *FGFR3*-related skeletal dysplasias

EITHER

1. **A parent has an *FGFR3*-related skeletal dysplasia**

OR

2. **A previous pregnancy has been confirmed to have an *FGFR3*-related skeletal dysplasia, thus there is a very small risk of recurrence due to germline mosaicism**

OR

3. **Abnormal ultrasound findings compatible with a sonographic diagnosis of *FGFR3*-related skeletal dysplasia**
    a. For achondroplasia and other rarer forms of FGFR3-related skeletal dysplasia (including Muenke syndrome, hypochondroplasia and hypochondroplasia plus acanthosis nigricans) the following features **must** be present:
        i. Femoral length on or above the 3rd percentile (i.e. within the normal range) at the routine 18-20 week scan
        ii. Femur length and all long bones below the 3rd percentile after 25 weeks gestation
        iii. Head circumference and abdominal circumference within or above the normal range for gestation at diagnosis, fetal and maternal dopplers should be normal

    Additionally, at least one of the following secondary features **should** be present:
        iv. Relative macrocephaly
        v. Frontal bossing
        vi. Short fingers

Figure 1. Testing criteria for NIPD for *FGFR3*-related skeletal dysplasias in the UK.