

## **Reproductive genetic screening for information: evolving paradigms?**

Reproductive genetic screening has introduced the possibility for pregnant women to learn, during the pregnancy or sometimes earlier, about the likelihood of their baby being affected with certain genetic conditions. As medicine progresses, the options afforded by this early information have expanded. This has led to a shifting paradigm in prenatal screening, wherein the early knowledge is seen as useful not only for its inherent value to the pregnant woman, but as enabling an expansion of conditions whose identification may allow early intervention and clinical impact. As the landscape of prenatal screening tests evolves, and the list of available target genetic conditions increases, it is worthwhile to evaluate what can be accomplished with today's technology. There are three interesting areas of reproductive-related screening which will be considered here: expanded carrier screening, non-invasive prenatal screening for aneuploidies and selected microdeletions, and non-invasive prenatal screening for single gene disorders.

### **What conditions do we want to screen for?**

Trisomy 21 forms part of a routine offer of prenatal screening in a number of countries. Trisomy 21 was one of the first genetic conditions for which a cytogenetic cause was identified and it became possible to identify this prenatally using amniocentesis and karyotyping even in the early days of cytogenetics (Valenti et al 1968). The association between altered levels of various serum markers and likelihood of trisomy 21 then allowed screening to be carried out to refine the risks for a pregnancy before offering amniocentesis (Driscoll 2004). This offering eventually expanded to include trisomies 13 and 18. However, these three trisomies only represent a small proportion of all of the genetic conditions which may affect an individual. Therefore, there is an argument that prenatal screening options should be expanded to include conditions that fulfill a considered set of criteria that make them appropriate for prenatal screening. Such criteria could include, for example, prevalence, penetrance, severity of phenotype and conditions where early knowledge could affect management and have the potential to improve outcomes. This is particularly pertinent at this point in time when gene therapies are becoming available for a selection of genetic conditions. In general, the earlier a medical issue is identified and treatment is started, the better the likelihood of an improved outcome. With genetic conditions such as spinal muscular atrophy this difference can be dramatic: without specific treatment, the progression of the disease can be rapid and devastating, whereas with early treatment the child may achieve milestones that would have been unthinkable previously (Messina 2020).

Additionally, early information is valuable for a number of other reasons:

1. Information given in pregnancy allows the woman or couple time to prepare and to plan for the birth of a child who may have difficulties. This planning could be practical, such as meeting doctors who will be involved in their child's care, or contacting associations, it could be financial, particularly in systems where there is no or limited public health care available, and it could be psychological, for example coming to terms with altered expectations and preparing the wider social circle for the new arrival.
2. In some circumstances the pregnant woman or couple may decide, having learned about the presence of a genetic condition in the fetus, that they will not continue with the

pregnancy. Learning about the condition during pregnancy is the only way that they would have the option to exercise this aspect of reproductive autonomy.

3. For certain conditions, preimplantation genetic testing is available, involving undergoing IVF and testing embryos prior to implantation for a known genetic condition in the family.
4. For some conditions, immediate postnatal interventions are required for optimal outcomes, but appropriate screening tests may not be routinely available after birth, or results may only be available days or weeks after birth. In these cases, prenatal testing is the only way to ensure that these interventions are available in a timely manner. For example there is an expression that 'time is motor neurons' (Govoni 2018), referring to the importance of and advantage of early identification and commencement of treatment to maximise the benefits of knowledge and appropriate management).

### **Carrier screening**

Carrier screening, which is designed to identify genetic variants in prospective parents that are associated with affected children, forms part of the prenatal screening paradigm as it can give information about the risk of passing on a genetic condition. It may be the only opportunity of finding out of such risk ahead of the birth of an affected child, as most of the conditions detectable by carrier screening are not screened for by routine prenatal screening. These conditions affect single genes rather than chromosomes. Common conditions seen on carrier screens include spinal muscular atrophy or cystic fibrosis. It has been shown that as many as 88% of carriers of cystic fibrosis, spinal muscular atrophy and fragile X syndrome have no known family history of the condition (Archibald et al 2018). Carrier screening has traditionally been offered on the basis of ethnicity, so would be offered for specific conditions to groups known to be at increased risk of those conditions, for example Tay-Sach's disease in the Ashkenazi Jewish population which was first introduced in the 1970's (Burke et al 2011). However there is increasing awareness that such an approach can miss a significant proportion of carriers, reflected in recent ACOG guidelines to offer screening for certain conditions, including cystic fibrosis and spinal muscular atrophy, to all patients who are pregnant or considering pregnancy regardless of ethnicity (ACOG). The possibility of missing carriers with non-typical ethnicities for the condition applies even for conditions that have been strongly associated with particular populations. This has come to light partly because the advent of more widely available screening has made it possible to find carriers in populations that would previously not have been offered testing (Westemyer et al 2020). As ancestry DNA tests are showing, people may not be fully aware of the specific risk groups they may belong to, so they would not be offered appropriate screening for their ethnic background (Shraga 2017).

The value of carrier screening can be shown through the example of spinal muscular atrophy (SMA) screening. SMA, one of the most common autosomal recessive diseases, is a progressive neurodegenerative condition which is associated with muscle weakness and respiratory difficulties (Messina 2020). It is due to a deletion of the SMN 1 gene (Messina 2020). The carrier rate is around 1 in 50, and this is a pan-ethnic condition (Sugerman 2012). Until recently, the management of this condition was primarily supportive, but there have been two major developments. The first is a medication called Nusinersen, an antisense oligonucleotide that

binds RNA. This targets a gene (SMN2), which is very similar to SMN1 and causes it to function more like SMN1 (Li 2020). It has been shown to be associated with improvements in motor milestones and a reduction in mortality and there was indication that the efficacy might be improved by early therapy initiation (Li 2020). Further developments have led to the development of a gene therapy for SMA (Messina 2020). Again data indicates that very early treatment is associated with better outcome (Messina 2020). Another example is cystic fibrosis, which now has a number of treatments available, including 4 designed to help to restore gene function (<https://www.cff.org/Life-With-CF/Treatments-and-Therapies/Medications/CFTR-Modulator-Therapies/> Accessed April 2020). Duchenne Muscular Dystrophy, another example, also has an increasing number of therapies that target gene function, and gene therapy trials are underway ( Flotats-Bastardas 2020).

### **Why screen for a condition that has treatments available?**

One question that could be asked is ‘why should we offer carrier screening for conditions where treatments are available?’. A goal of prenatal screening is to identify medical conditions to help to make choices regarding care and treatment during the pregnancy or after the baby is born (Public Health England). Carrier screening offers this, and in fact goes further, providing information that allows couples to make decisions at every stage of the reproductive process. Carrier screening offers couples the opportunity to have information before even becoming pregnant, about what their chances might be of having a baby with certain specific genetic conditions. This allows them to decide whether they wish to progress with pregnancy naturally or whether to select other options that may be available to them such as preimplantation genetic diagnosis, adoption or egg/sperm donation. If they do opt for natural pregnancy, they then have the option to confirm whether or not their baby will inherit the condition. Having this knowledge then gives them the opportunity to decide whether or not to continue the pregnancy and if so, to have the chance to prepare themselves and others. It also gives the opportunity for the baby to be given appropriate treatment at the earliest possible opportunity. Not all conditions screened for by carrier screening are currently offered as routine postnatal screens, and in some places no or little postnatal screening is available. It could thus be weeks, months or years before the condition is detected.

If we accept that having the option to obtain information about important potential genetic conditions in the fetus is a valuable endeavor, then carrier screening can be considered an option that women and their partners should be informed about, ideally before pregnancy, and in fact this is the recommendation of the American College of Obstetrics and Gynecology(ACOG 2017).

### **Expanding the offer of non-invasive prenatal genetic screening**

In 2011 tests using cell-free DNA, known as ‘non-invasive prenatal screening’ (NIPS) became available to screen for trisomy 21 (Palomaki et al 2011). This approach represents a significant advance: rather than using proxy markers, DNA originating directly from the pregnancy is assessed for the genetic evidence of the additional chromosome material that is the hallmark of trisomy (Palomaki et al 2011). Although this technique targets the DNA that defines the condition, it is considered a screening test rather than diagnosis. One reason for this is that it is looking at cell-free DNA from the placenta and not directly from the fetus, and confined

placental mosaicism can confound results (Grati et al 2013). Regardless, a head to head comparison of NIPS and standard first trimester screening incorporating maternal serum markers demonstrated significantly superior performance for NIPS for trisomy 21 (Norton et al 2015).

More recently, some NIPS tests added a set of key microdeletions to their offering (Wapner 2015), and some NIPS have started offering genome-wide screening for large, unselected copy number variants (Van der Meij et al 2019). If we adhere to the principles for prenatal screening outlined above, we might consider the addition of at least some microdeletions to the offer of prenatal genetic screening. Let us consider the case for microdeletion 22q11.2, now offered as part of several NIPS tests. This condition is the most common human microdeletion syndrome. Studies have indicated an incidence of between 1/1000 to 1/6000 (Meisenbacher et al 2017), although the true prevalence is unknown as not all cases are detected. This is a serious condition which is associated with congenital cardiac defects, developmental delay and learning difficulties, immune deficiencies, palate malformations, hypocalcaemia and psychiatric disorders among other clinical features (McDonald-McGinn et al 2020). The condition is variable, with some individuals being severely affected whereas others may be mildly or asymptomatic, but penetrance is complete in the majority of cases (McDonald-McGinn et al 2020). Whilst performance rates will vary according to the specific test used, detection rates for the common 2.91Mb deletion of 90% have been published for the SNP NIPS technology (Ravi et al 2018), and a false positive rate of 0.07% (Martin et al 2018). It is important when thinking of expanding prenatal genetic screening to consider false positive rates, which are higher for copy number variants than for the common trisomies.

The advantages of prenatal screening for 22q include early information for parents and healthcare professionals to manage the pregnancy and prepare for the birth and potential health issues. In the immediate postnatal period, there are a number of interventions which may improve outcome, such as doing a detailed cardiac scan before the baby leaves hospital, and checking for hypocalcaemia, immune deficiency, and palatal anomalies (Ravi et al 2018). Early intervention in speech and motor development, which has been advised to start before 12 months if possible (Gerdes et al 2001) can be put into place. Children with undiagnosed 22q11 deletion may undergo a diagnostic odyssey in which multiple healthcare providers are consulted before an underlying explanation for the various developmental and health issues is found, and again prenatal screening could help to avoid this and maximise opportunities for early and appropriate management (Ravi et al 2018). It is important to note that counselling for microdeletion 22q screening should include the fact that this is a variable condition, the severity of which cannot be determined in advance on an individual level, and that around 10% of cases are inherited (McDonald-McGinn et al 2020). A parent may therefore find out that they also have 22q11 deletion. This in turn has benefits as they can seek appropriate surveillance and also be given more accurate information about the likelihood of 22q11 in future pregnancies (50% for affected parents).

### **Single gene testing**

One of the most recent advances in NIPS is the possibility to offer non-invasive prenatal screening for some monogenic conditions (Zhang et al 2019). Many of these would not be detected by routine prenatal screening, may be detected late, or involve non-specific ultrasound

findings. Early knowledge of the likelihood of such a condition may help to avoid uncertainty when non-specific signs are detected, and to inform about the likelihood of a condition for which no ultrasound signs would be present, such as Rett syndrome (Fu et al 2020). This information may guide pregnancy and delivery management and care in the neonatal period and beyond. This type of testing may be of particular interest when there is advanced paternal age (generally considered to be 40 or above) (Nwakalor et al 2021), where there are non-specific ultrasound signs and diagnostic testing is unavailable or is refused by the couple, as well as prospective parents who want to increase the range of conditions that they are screening for in pregnancy.

## Conclusions

Prenatal genetic screening has developed rapidly over the past decade. As it becomes possible to screen for more conditions, it is important to reflect on the risks and benefits of adding each condition. Just as it would be wrong to add a condition purely because it is technically possible, it is also important to review whether there are additional conditions which should be included, for the value of the information itself and for the clinical actionability. As genetic screening technology advances, there will be more options for prenatal genetic screening, which offer exciting opportunities to improve outcomes through early diagnosis and appropriate management. A well-thought-out offer of screening can include different types of genetic conditions – selected whole chromosome conditions, copy number variants and single gene changes, with the aim of maximizing the availability of useful information. Appropriate counselling is vital to help to support prospective parents to understand their options and to make informed decisions about what screening is right for them.

## REFERENCES

ACOG. 2017. Committee Opinion: Carrier screening in the age of genomic medicine.

Burke W, Tarini B, Press NA, Evans JP. Genetic screening. *Epidemiol Rev.* 2011;33(1):148-64. doi: 10.1093/epirev/mxr008. Epub 2011 Jun 27. PMID: 21709145; PMCID: PMC3166195.

CFF.org [https://www.cff.org/Life-With-CF/Treatments-and-Therapies/Medications/CFTR-Modulator-Therapies/Accessed April 2020](https://www.cff.org/Life-With-CF/Treatments-and-Therapies/Medications/CFTR-Modulator-Therapies/Accessed%20April%202020)

Driscoll DA; Professional Practice and Guidelines Committee. Second trimester maternal serum screening for fetal open neural tube defects and aneuploidy. *Genet Med.* 2004 Nov-Dec;6(6):540-1. doi: 10.1097/00125817-200411000-00013. PMID: 15545752.

Flotats-Bastardas M, Hahn A. New Therapeutics Options for Pediatric Neuromuscular Disorders. *Front Pediatr.* 2020 Nov 23;8:583877. doi: 10.3389/fped.2020.583877. PMID: 33330280; PMCID: PMC7719776.

Fu C, Armstrong D, Marsh E, Lieberman D, Motil K, Witt R, Standridge S, Nues P, Lane J, Dinkel T, Coenraads M, von Hehn J, Jones M, Hale K, Suter B, Glaze D, Neul J, Percy A, Benke T. Consensus guidelines on managing Rett syndrome across the lifespan. *BMJ Paediatr Open.*

2020 Sep 13;4(1):e000717. doi: 10.1136/bmjpo-2020-000717. PMID: 32984552; PMCID: PMC7488790.

Gerdes M, Solot C, Wang PP, McDonald-McGinn DM, Zackai EH. Taking advantage of early diagnosis: preschool children with the 22q11.2 deletion. *Genet Med*. 2001 Jan-Feb;3(1):40-4. doi: 10.1097/00125817-200101000-00009. PMID: 11339376.

Govoni A, Gagliardi D, Comi GP, Corti S. Time Is Motor Neuron: Therapeutic Window and Its Correlation with Pathogenetic Mechanisms in Spinal Muscular Atrophy. *Mol Neurobiol*. 2018 Aug;55(8):6307-6318. doi: 10.1007/s12035-017-0831-9. Epub 2018 Jan 2. PMID: 29294245.

Kraft SA, Duenas D, Wilfond BS, Goddard KAB. The evolving landscape of expanded carrier screening: challenges and opportunities. *Genet Med*. 2019 Apr;21(4):790-797. doi: 10.1038/s41436-018-0273-4. Epub 2018 Sep 24. PMID: 30245516; PMCID: PMC6752283.

Li Q. Nusinersen as a Therapeutic Agent for Spinal Muscular Atrophy. *Yonsei Med J*. 2020 Apr;61(4):273-283. doi: 10.3349/ymj.2020.61.4.273. PMID: 32233169; PMCID: PMC7105407.

Martin K, Iyengar S, Kalyan A, Lan C, Simon AL, Stosic M, Kobara K, Ravi H, Truong T, Ryan A, Demko ZP, Benn P. Clinical experience with a single-nucleotide polymorphism-based non-invasive prenatal test for five clinically significant microdeletions. *Clin Genet*. 2018 Feb;93(2):293-300. doi: 10.1111/cge.13098. Epub 2017 Nov 17. PMID: 28696552.

McDonald-McGinn DM, Hain HS, Emanuel BS, Zackai EH. 22q11.2 Deletion Syndrome. 1999 Sep 23 [updated 2020 Feb 27]. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, editors. *GeneReviews*<sup>®</sup> [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2020. PMID: 20301696.

Messina S, Sframeli M. New Treatments in Spinal Muscular Atrophy: Positive Results and New Challenges. *J Clin Med*. 2020 Jul 13;9(7):2222. doi: 10.3390/jcm9072222. PMID: 32668756; PMCID: PMC7408870.

M Maisenbacher MK, Merrion K, Pettersen B, Young M, Paik K, Iyengar S, Kareht S, Sigurjonsson S, Demko ZP, Martin KA. Incidence of the 22q11.2 deletion in a large cohort of miscarriage samples. *Mol Cytogenet*. 2017 Mar 9;10:6. doi: 10.1186/s13039-017-0308-6. PMID: 28293297; PMCID: PMC5345148.

Norton ME, Jacobsson B, Swamy GK, Laurent LC, Ranzini AC, Brar H, Tomlinson MW, Pereira L, Spitz JL, Hollemon D, Cuckle H, Musci TJ, Wapner RJ. Cell-free DNA analysis for noninvasive examination of trisomy. *N Engl J Med*. 2015 Apr 23;372(17):1589-97. doi: 10.1056/NEJMoa1407349. Epub 2015 Apr 1. PMID: 25830321.

Nwakalor C, Said-Delgado S, Krinshpun S, Velinov M. De novo . HRAS gene mutation associated with Costello syndrome identified by non-invasive cell-free fetal DNA screening. *Prenat Diagn.* 2021 Jan;41(1):11-14. doi: 10.1002/pd.5798. Epub 2020 Sep 9. PMID: 32681669.

Palomaki GE, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, Bombard AT, Deciu C, Grody WW, Nelson SF, Canick JA. DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet Med.* 2011 Nov;13(11):913-20. doi: 10.1097/GIM.0b013e3182368a0e. PMID: 22005709.

Public Health England Screening tests for you and your baby.  
<https://www.gov.uk/government/publications/screening-tests-for-you-and-your-baby/introduction>. Accessed 13 March 2021.

Ravi H, McNeill G, Goel S, Meltzer SD, Hunkapiller N, Ryan A, Levy B, Demko ZP. Validation of a SNP-based non-invasive prenatal test to detect the fetal 22q11.2 deletion in maternal plasma samples. *PLoS One.* 2018 Feb 23;13(2):e0193476. doi: 10.1371/journal.pone.0193476. PMID: 29474437; PMCID: PMC5825123.

Shraga R, Yarnall S, Elango S, Manoharan A, Rodriguez SA, Bristow SL, Kumar N, Niknazar M, Hoffman D, Ghadir S, Vassena R, Chen SH, Hershlag A, Grifo J, Puig O. Evaluating genetic ancestry and self-reported ethnicity in the context of carrier screening. *BMC Genet.* 2017 Nov 28;18(1):99. doi: 10.1186/s12863-017-0570-y. PMID: 29179688; PMCID: PMC5704547.

Sugarman EA, Nagan N, Zhu H, Akmaev VR, Zhou Z, Rohlfes EM, Flynn K, Hendrickson BC, Scholl T, Sirko-Osadsa DA, Allitto BA. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of >72,400 specimens. *Eur J Hum Genet.* 2012 Jan;20(1):27-32. doi: 10.1038/ejhg.2011.134. Epub 2011 Aug 3. PMID: 21811307; PMCID: PMC3234503.

Valenti C, Schutta EJ, Kehaty T. Prenatal diagnosis of Down's syndrome. *Lancet.* 1968 Jul 27;2(7561):220. doi: 10.1016/s0140-6736(68)92656-1. PMID: 4173436.

van der Meij KRM, Sistermans EA, Macville MVE, Stevens SJC, Bax CJ, Bekker MN, Bilardo CM, Boon EMJ, Boter M, Diderich KEM, de Die-Smulders CEM, Duin LK, Faas BHW, Feenstra I, Haak MC, Hoffer MJV, den Hollander NS, Hollink IHIM, Jehee FS, Knapen MFCM, Kooper AJA, van Langen IM, Lichtenbelt KD, Linskens IH, van Maarle MC, Oepkes D, Pieters MJ, Schuring-Blom GH, Sikkel E, Sikkema-Raddatz B, Smeets DFCM, Srebniak MI, Suijkerbuijk RF, Tan-Sindhunata GM, van der Ven AJEM, van Zelder-Bhola SL, Henneman L, Galjaard RH, Van Opstal D, Weiss MM; Dutch NIPT Consortium. TRIDENT-2: National Implementation of Genome-wide Non-invasive Prenatal Testing as a First-Tier Screening Test in the Netherlands. *Am J Hum Genet.* 2019 Dec 5;105(6):1091-1101. doi: 10.1016/j.ajhg.2019.10.005. Epub 2019 Nov 7. PMID: 31708118; PMCID: PMC6904791.

Wapner RJ, Babiarz JE, Levy B, Stosic M, Zimmermann B, Sigurjonsson S, Wayham N, Ryan A, Banjevic M, Lacroute P, Hu J, Hall MP, Demko Z, Siddiqui A, Rabinowitz M, Gross SJ, Hill M, Benn P. Expanding the scope of noninvasive prenatal testing: detection of fetal microdeletion syndromes. *Am J Obstet Gynecol.* 2015 Mar;212(3):332.e1-9. doi: 10.1016/j.ajog.2014.11.041. Epub 2014 Dec 2. PMID: 25479548.

Westemeyer M, Saucier J, Wallace J, Prins SA, Shetty A, Malhotra M, Demko ZP, Eng CM, Weckstein L, Boostanfar R, Rabinowitz M, Benn P, Keen-Kim D, Billings P. Clinical experience with carrier screening in a general population: support for a comprehensive pan-ethnic approach. *Genet Med.* 2020 Aug;22(8):1320-1328. doi: 10.1038/s41436-020-0807-4. Epub 2020 May 5. Erratum in: *Genet Med.* 2020 Jun 2;: PMID: 32366966; PMCID: PMC7394882.

Zhang J, Li J, Saucier JB, Feng Y, Jiang Y, Sinson J, McCombs AK, Schmitt ES, Peacock S, Chen S, Dai H, Ge X, Wang G, Shaw CA, Mei H, Breman A, Xia F, Yang Y, Purgason A, Pourpak A, Chen Z, Wang X, Wang Y, Kulkarni S, Choy KW, Wapner RJ, Van den Veyver IB, Beaudet A, Parmar S, Wong LJ, Eng CM. Non-invasive prenatal sequencing for multiple Mendelian monogenic disorders using circulating cell-free fetal DNA. *Nat Med.* 2019 Mar;25(3):439-447. doi: 10.1038/s41591-018-0334-x. Epub 2019 Jan 28. Erratum in: *Nat Med.* 2019 Feb 20;: PMID: 30692697.