Invited Commentary for 6 in 60, Journal of Medical Genetics

“Have you tested for 22q?”

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This paper was a multicentre study aimed at a survey of the clinical phenotypes associated with deletion of 22q11.2 (22q11.2DS)[1]. At the outset of the study it was well known, in clinical genetic circles at least, that such deletions were present in patients previously diagnosed with specific diagnostic labels. Most commonly these were DiGeorge (MIM#188400) or velocardiofacial (MIM#192430) syndromes, but also included Cayler asymmetric crying facies (MIM%125520), conotruncal anomaly face, Sedlackova, and a small subset of Opitz GBBB syndromes (these latter having no separate OMIM entry). Molecular diagnosis was required to detect deletions in the vast majority of cases using microsatellite analysis fluorescence *in situ* hybridization (FISH), or both, with probes freely available to requesting laboratories.

Over 25 years on, this 6 in 60 commentary gives an opportunity to ask how the paper has stood the test of time. Additionally, I describe briefly where this study, and similar research conducted elsewhere, has led us over the subsequent quarter century.

The single major advantage of the collaboration paper was the number of patients included in the report, 558. Of course, it was a retrospective study subject to the

constraints of ascertainment bias, partial information and differing medical nomenclature used between international centres. Dr. Anna Ryan, a clinical fellow with Prof. John Burn in Newcastle, made best sense of these, handled the database and follow on queries. I have fond memories of collaborating with the Newcastle team, which started just as soon as we had molecular tools to work with.

Reading the paper today, the main findings are consistent with current knowledge. They emphasize the high rate of congenital heart defect as causing presenting disease, especially early in life, and the protean nature of the condition with variation between sibs or between parent and child, or both. The suggested paternal origin of de novo deletions (Table 1) is incorrect (indeed we cited a contradictory publication in the discussion). Growth parameters should have been much better analysed, although longitudinal data was almost entirely unavailable. While DiGeorge syndrome was in the textbooks as an immunodeficiency syndrome we were correct that, while a cause of morbidity, immunodeficiency was not a major cause of mortality for the condition. We also highlighted the range of behavioural and neurological presentations, although again we had no information on the evolution of these presentations. The incidence of gastrointestinal issues was underestimated. As our study did not go back to the families, we had little inkling of what they saw as the major issues at each stage of a patient's life (e.g. sleep problems, feeding, schooling, transition to adulthood). That has been remedied over the years, with studies also aimed specifically at understanding how family life is affected, for example mental health issues in mothers [2].

An independent study also published in 1997 came from Children's Hospital of Philadelphia and had the singular advantage that patients were followed by a single clinician from that centre [3]. In the main, this study had far superior neurological

assessments. (As an aside, this centre has subsequently reported on over 1400 cases[4]). Together these papers present a decent image of the syndrome as a whole, one to which various sub-specialists have subsequently added much refinement. Clinical geneticists, by 1997, were well aware of the syndrome: I recall several London Dysmorphology Club meetings of the mid-90s (run by Di Donnai and Robin Winter) where “Have you looked at 22q?” was a constant refrain for an “unknown” [diagnosis] that had any degree of clinical overlap with 22q11.2DS. Through publication, conferences and the work of the various patient/family support groups awareness in paediatric and other specialties has improved markedly. The difficulties faced by families in obtaining a diagnosis of 22q11.2DS was termed their “diagnostic odyssey” (Figure 1)[5]. As well as awareness amongst clinicians, support groups are imaginatively targeting the general public with international initiatives such as 22q at the Zoo (https://22q.org/get-involved/22q-at-the-zoo/), and "Luces por el 22q/Lighting the 22q" (https://22q11europe.org/22q11-day-europe-lights-up-red/), held on the 22nd November (get it?). The UK even has an All Party Parliamentary Group (APPG) to promote interests of patients and families within the National Health Service.

The year following these two clinical surveys the first international meeting on 22q11DS was held in Strasbourg. This initiated a remarkable period of collaboration at both the clinical and basic research levels with a series of further biennial meetings held in North America and Europe. Family meetings were sometimes held as overlapping satellites, with support groups, patients and families present at all. Current meetings are organized under the auspices of the 22q11.2 Society (originally a UK charity, now based in the US; https://22qsociety.org/). The 13th such meeting was held near Obidos, Portugal. A sample range of presentations gives an

idea of the breadth of current research: perturb-seq as a means of examining the role of multi(22q)gene knockdown in brain organoids; cardiac organoids in 22q11.2DS modelling; a state-of-the art overview of CNVs; non-invasive screening for 22q11DS during pregnancy; scoliosis; leg pain; dental caries, surgical decision making in velopharyngeal dysfunction; atypical 22q11.2 deletions; 22q11.2 duplications. Research updates were given from ongoing consortia such as the genetic modifier group (examining genotype-phenotype relationships) and the IBBC (International Brain Behavior Consortium). This latter was instituted because of the high risk of psychiatric illness and behavioural difficulty in the syndrome, with 22q11.2 often cited as a good model for probing the biological underpinnings of functional psychosis[6][7].

Brief survey of current knowledge

More detailed clinical (as well as basic science) surveys are available in a recently published book on the syndrome, as near as one can get to a one stop state of the art summary [8]. We now have considerable information concerning the protein-encoding genes (~50), long non-coding RNAs (lncRNAs), and microRNAs (miRNAs) reduced to hemizygosity by the most common 3Mb deletion (85-90% of cases). TBX1, a transcription factor, is especially important for heart, thymus, parathyroid and ear development [9] . Heart defects in particular are likely impacted by CRKL, a signalling adapter[10]. DGCR8, a protein required for RNA processing[11], is implicated in several neurological phenotypes.

The search for explanations of the variable phenotype is only partially answered. There is a minor contribution from the size of deletion (e.g. with or without deletion of CRKL[10], rare variants elsewhere in the genome (e.g. involving chromatin

modifiers[12]), and extremely rare variants on the non-deleted chromosome that result in a concomitant recessive disease. Examples of this include (affected gene in parentheses) Bernard-Soulier (MIM#231200) (*GP1BB*), Hyperprolinaemia type 1 (MIM#239500) (*PRODH*), CEDNIK (MIM#609528) (*SNAP29*), Ven den Edna Gupta (MIM#600920)(*SCARF2*), autosomal recessive Noonan (MIM#605275) (*LZTR1*), D-2-, L-2-hydroxyglutaric aciduria (*SLC25a1*) (MIM#615182), CGS (no OMIM designation) (*CDC45*)(possibly a variant of Meier-Gorlin 7 syndrome (MIM#617063)), and MECRCN (MIM#616878) (*TANGO2*) syndromes.

Deletions are caused by non-allelic homologous recombination between low-copy repeat sequences (LCRs, [13]) which may also result in the reciprocal duplication. In general, the duplication syndrome (MIM#608363) has a much milder phenotype that the deletion, but also includes cardiac anomalies, velopharyngeal insufficiency and psychomotor retardation [14] . Remarkably, some duplication cases were detected due to suspicion of 22q11.2 deletion. Recombination at different LCRs may produce variable deletions distal to the more frequent proximal abnormalities[15] i.e. involving distinct sets of genetic hemizygosity (MIM#611867). The phenotype may again involve congenital heart defect.

Clinicians now have access to a series of best practice guidelines for children [16] and adults with advice on managing the switch from paediatric to adult care [17, 18] and how patient needs are likely to evolve with age [18]collated by members of the 22q11.2 Society. As the basic science helps us understand more about specific pathways affected downstream of the deletion it is possible to speculate about what new treatments could look like. On the one hand, highly technological interventions could target specific genes or pathways in required cells or tissues, but are likely to be expensive. On the other, small molecules, metabolites and even nutrients may

make small but useful improvements (e.g. vitamin B12 megadoses improve some *Tbx1* null embryonic phenotypes in mice [19]). Even given the relatively high birth incidence (versus other rare diseases) one issue is how to trial any potential treatment given the number of candidate interventions likely to be proposed and the relatively small number of patients available without one trial confounding another. Hopefully, the use of iPSCs, embryoids and organoids will help to narrow and prioritize the field of candidates.

Should we screen for 22q11.2 deletions?

Both current and future treatments are likely to be improved by early diagnosis. The median age at diagnosis is at one year old. Given the mild phenotype of some individuals with 22q11.2 deletions it remains the case that the deletions go undetected. Attempts at deriving incidence figures come from screening studies. One examined over 30,000 dried blood spots over a 21 month period and found deletions in 1 of every 2148 live births [20]. Another found an incidence of ~1:1000 in a “low risk” obstetric population, with a similar incidence of duplication of 22q11.2[21]. Even if “low risk” is not representative of the general population, taken together these studies suggest 22q11.2DS should have a high priority for inclusion in current neonatal screening programs, and future multiplex prenatal screens.

Acknowledgements

Such a study would have been impossible without patient and family co-operation and clinicians who referred patients over the years. The 1997 study was largely funded via the EU, but the British Heart Foundation, Medical Research Council and Newlife made major contributions to my work on the syndrome. Finally, I am indebted to my research team and collaborators.

Conflict of Interest

None declared.

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FIGURE LEGEND

Figure 1

The elephant logo of the 22q11.2 Society was inspired by the diagnostic odyssey faced by patients and families before the deletion syndrome was widely recognised. Patients would be referred to different clinicians depending upon the issue presenting at the time, often resulting in a failure to join the dots, make a syndromic diagnosis and institute appropriate care pathways.