POSITION STATEMENT

Structural Aberrations with Secondary Implications (SASIs): consensus recommendations for reporting of cancer susceptibility genes identified during analysis of Copy Number Variants (CNVs)

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ABSTRACT

Clinical testing with chromosomal microarray (CMA) is most commonly undertaken for clinical indications such as intellectual disability, dysmorphic features and/or congenital abnormalities. Identification of a structural aberration (SA) involving a cancer susceptibility gene (CSG) constitutes a type of incidental or secondary finding. Laboratory reporting, risk communication and clinical management of these structural aberrations with secondary implications (SASIs) is currently inconsistent. We undertake meta-analysis of 18 622 instances of CMA performed for unrelated indications in which 106 SASIs are identified involving in total 40 different CSGs. Here we present the recommendations of a joint UK working group representing the British Society of Genomic Medicine, UK Cancer Genetics Group and UK Association for Clinical Genomic Science. SASIs are categorised into four groups, defined by the type of SA and the cancer risk. For each group, recommendations are provided regarding reflex parental testing and cancer risk management.

INTRODUCTION

In widespread use in diagnostic genetic laboratories are genome-wide tests for the identification of structural aberrations (SAs), in particular copy number gains and losses at the chromosomal level (Copy Number Variants (CNVs)). Chromosomal microarray (CMA) is currently the most widely used approach but clinical application of whole genome sequencing (WGS) and whole exome sequencing is expanding rapidly. Currently, these tests are most frequently undertaken to investigate intellectual disability, dysmorphic features and/or congenital abnormalities detected in childhood or prenatally. However, a SA identified on CMA often encompasses many genes. When a medically relevant gene, unrelated to the primary indication for testing, is identified within a SA, this constitutes a form of incidental or secondary finding.[1] We hereafter refer to a SA containing an incidentally detected medically relevant gene as a structural aberration with secondary implications (SASI; see the Glossary in supplementary file 1).

Current clinical laboratory management of SASIs varies widely with regard to (1) The degree to which SAs, once detected, are interrogated for the presence of medically relevant genes. (2) Which medically relevant genes are reported when found within a putative primary (causative) SA. (3) Information given regarding elevation of disease risk and recommended management. With widening implementation of genomic technology across healthcare and expansion of WGS, consistency of approach is urgently required.

On account of these disparities in practice, a UK working group was convened to deliver a consensus UK framework for management of SASIs, focusing in the first instance on SASIs involving cancer susceptibility genes (CSG-SASIs).

APPROACH AND METHODS

Selected representatives from the British Society of Genomic Medicine, the UK Association for Clinical Genomic Science and the UK Cancer Genetics Group were nominated for membership of the working group. Six meetings were convened to:

- Identify issues relevant to development of a framework for analysis and reporting of CSG-SASIs.
- Define which CSGs should be included as being medically relevant for analyses of SASIs.
- Explore the frequency of involvement of CSGs in (1) Recognised SA-related syndromes. (2) Other SAs.
- Explore penetrance for cancer associated with CSG-SASIs. Literature review was undertaken to assess disease association and genotype-phenotype correlations using PubMed, Web of Science, OMIM (Online Mendelian Inheritance in Man) and Human Gene Mutation Database.[3 4]

RESULTS

(1) Issues considered relevant to development of a framework on analysis and reporting of CSG-SASIs.

Context of testing

Although most commonly performed in the paediatric setting, CMA is increasingly undertaken in the prenatal setting to investigate abnormalities

identified on prenatal imaging. In the prenatal context, particularly, the tone and scope of how a medically relevant gene is reported, including those additional and unrelated to the presenting feature(s), may contribute towards the binary irreversible decision of whether to terminate the pregnancy.

Types of Structural Aberrations (SAs)

For genes acting via loss of function (LOF), it is predicted that whole or partial gene loss will likely have effects equivalent to those of a classic protein-truncating pathogenic sequence variant. Intragenic duplications (resulting in nonsense-mediated decay) are also predicted to result in a null allele (hereafter termed 'within-gene duplications'). More commonly detected on CMA are 'large duplications' which fully encompass a gene; these are predicted to be less impactful with regard to functions of the genes encompassed within. For genes acting via gain of function (GOF), the implications on gene function of CMA-detected deletions or duplications are less readily predicted.

'Clinical Utility' of medically relevant genes

Several thousand gene-disease associations have been reported.[3 4] However, for only a small minority of these is (1) The associated disease perceived to be 'severe'. (2) The penetrance established as 'moderate' or 'high'. (3) Variant pathogenicity and genotype-phenotype correlations well understood. (4) Available interventions proven to be clinically effective and/or of acceptable risk-benefit.[5 6] Judged against such criteria, a modest number of genes have been designated as being of sufficient 'clinical utility', that, regardless of the medical condition for which the patient was investigated, offer of interrogation for pathogenic variants as 'secondary findings' has been recommended. These we hereafter refer to as 'high-actionability' genes.

Penetrance

The majority of available data on penetrance for disease are derived from cases ascertained in the context of a 'classic' phenotype and/or relevant family history. A number of recent analyses suggest that, likely through enrichment for genetic modifiers, these penetrance estimates are substantially upwardly biased compared with the 'average' or population-associated risk of pathogenic sequence variants in that gene.[7–9] Uncertainty regarding penetrance is a predominant concern countering more widespread return of pathogenic sequence variant 'secondary findings' detected on exome/WGS analysis.[10] A further tier of uncertainty pertains to CSG-SASIs, on account of the mutational mechanism also being different to that for which most penetrance estimates have been derived.

Age-related cancer penetrance

For different genes, the typical age at which associated cancers are anticipated to manifest varies from early childhood to late middle age.

Efficacy of available interventions

For many rare genetic disorders involving elevated susceptibility to cancer, there is sparse evidence regarding clinical effectiveness of the interventions widely adopted for prevention and/or screening, let alone robust health economic evaluation. Even for those gene-disease-intervention paradigms where the evidence base is more robust, the benefits of intervention are strongly predicated on the cancer penetrance of pathogenic variants. If penetrance is substantially lower than the figures used in prior evaluations of interventions, the cost-benefit profile will alter accordingly.

Contextualising clinical priorities

For children and adults with significant intellectual impairment and/or behavioural issues, some 'routine' screening investigations for cancer, such as MRI, may prove challenging, involve psychological distress, and/or necessitate the additional risk of sedation or anaesthesia. Furthermore, it is essential to contextualise a risk of cancer against the competing morbidities and mortality associated with their SA-related syndrome.

Autonomy, the right 'not-to-know' and longitudinal data management

It has been conventional practice in clinical genetics to offer testing for genetic findings relating to disorders of adult onset only when that patient has reached adulthood (or is deemed Gillick competent).[11] This protects the autonomy of decision-making for the individual concerned, including the right 'not-to-know'. However, prospective parents evaluating during pregnancy the overall lifetime 'prognosis' for their potential child, may deem relevant to that decision the presence of a CSG-SASI, even if relating to cancer risk of onset in late childhood or adulthood. Indeed, it is not unusual for parents to request prenatal testing or preimplantation genetic diagnosis if there is a pathogenic CSG sequence variant already known in the family.[12] Even if it were agreed preferable to withhold until adulthood such genetic information identified prenatally or in infancy, there is insufficient facility within current electronic patient record systems in the UK to guarantee the necessary robust longitudinal patient tracking.

Testing workflows, prenatal testing turnaround and parental consent

Interpretation of an SA deemed likely explanatory for the primary phenotype may involve testing of the parents. In particular, if the SA is of intermediate size and/or has not been previously reported, demonstration that the SA is de novo can constitute key evidence when assigning causality for the primary phenotype. Rapidity of CMA analysis, including parental analyses, is important in the investigation of paediatric cases, but is particularly critical in the prenatal context. For this reason, parental blood samples are often sought concurrent to sampling of the child/pregnancy, and generic consent is taken upfront for 'reflex' parental testing to be undertaken where required.

In most contexts, for a 'well' adult, 'predictive' testing for a pathogenic variant in a CSG is deemed worthy of detailed consideration and consultation with an experienced genetics professional is standard. This is because a positive genetic test result gives information about future cancer occurence for that well individual, in addition to potentially having additional implications for family members, reproduction and insurance.

Thus, expediency in reporting of prenatal tests must be balanced against the need for careful parental genetic counselling and avoidance of unsolicited 'predictive' testing.

(2) Which CSGs should be included in CSG-SASI analysis?

We defined CSGs as genes for which the relative risk for invasive cancer compared with the baseline population was >2–4 (intermediate penetrance) or >4 (high penetrance), and based on robust, reproducible genetic epidemiological data.[13] For these analyses we used a set of 115 CSGs, adapted from Rahman[14] (see online supplementary file 2).

To define high-actionability CSGs (HA-CSGs), we used the 23 CSGs which (1) Act through LOF. (2) Have autosomal dominant inheritance. (3) Are included in the American College of Medical Genetics (ACMG) list of 59 genes recommended for return of 'secondary' findings. Hereafter we refer to this group of genes as 'the ACMG 23 gene set'.[1 6 15 16] We note that for the UK 100,000 Genomes Project, a more conservative set of 10 CSGs has been used for secondary findings.[12] This currently includes, reported in adults only, *BRCA1*, *BRCA2*, *MLH1*, *MSH2*, *MSH6* and *MUTYH* (biallelic only) and, reported in both adults and children, *APC*, *MEN1*, *RET* and *VHL*.[17]

Recognised SA-related syndromes in which a CSG is involved

We used as 'established syndromes' involving an SA, the 67 syndromes described in the DECIPHER database (SA-related syndromes), of which 11 involve a CSG[2 18] (table 1). For only 2 of the 11 syndromes, review of the literature revealed clear evidence of a substantial elevation in lifetime cancer risk:

- (Micro)deletion at 5q21-q22, encompassing *APC*, are identified in approximately 15% of families investigated for early onset and/or familial polyposis.[19–21] Conversely, deletions of 5q21-q22 have also been identified in multiple individuals investigated for intellectual impairment, with subsequent elucidation on colonoscopic examination of highly penetrant polyposis characteristic of FAP (familial adenomatous polyposis).[22–24]
- (Micro)deletion at 11p13, encompassing *WT1*, is a frequent finding on investigation of children with Wilms' tumour, in particular when additional features are present.[25–27] Conversely, there is a clear increased prospective risk of Wilms' tumour (45%–60%) in children investigated for the aniridia, genitourinary abnormalities and/or growth and mental retardation characteristic of the **acronymous** WAGR syndrome in whom microdeletions of 11p13 are identified.[28 29]

For a further 2 of the 11 syndromes, there are a characteristic constellation of features, of which susceptibility to cancer is an established but not predominant element. For these, while (micro)deletion is a recognised mechanism causing LOF of the gene, clinical description of the phenotypical spectrum has largely been derived from studies of individuals with pathogenic sequence variants:

- (Micro)deletion at 17q11.2, encompassing *NF1,* are identified in ~5% of individuals investigated for clinical features of type 1 neurofibromatosis (NF1) and have a similar, if not more severe, phenotype to individuals with *NF1* pathogenic sequence variants.[30 31] Although predominantly associated with non-invasive tumours, individuals with NF1 have a risk of malignancy of 20% by age 50 years. The lifetime risk of malignant peripheral nerve sheath tumours is ~10% in NF1 and may be highest in patients with symptomatic NF1 with whole gene deletions.[32 33]
- (Micro)deletions at 5q35.3, encompassing *NSD1*, are identified in ~10% of non-Japanese cases of Sotos syndrome and are associated with more pronounced intellectual impairment and less marked overgrowth.[34 35] The estimated risk of paediatric malignancy in individuals with Sotos syndrome is 3% and includes sacrococcygeal teratomas and neuroblastoma.[36]

While there are no reports of classical Birt-Hogg-Dubé syndrome (BHD, MIM 135150) arising in cases of Smith-Magenis syndrome (MIM 182290), one individual with bilateral renal tumours aged 57 years has been reported in the literature.[37] However, this affected individual did not manifest other features of BHD nor were there additional pathological features supporting the renal cancers as being *FLCN*-associated rather than sporadic.[37] In a large series of molecularly characterised BHD cases, no such deletions at 17p11.2 were reported, with only small exon-level *FLCN* deletions described.[38]

There was no evidence of association with the respective cancer for the other seven (micro)deletion/duplication syndromes in table 1, based on literature review.

Of note, the typical 2.44 Mb 22q11 deletion (Velocardiofacial/Di George region; (DECIPHER GRCh37 22: 19 009 792–21 452 445)) contains *LZRT1,* a gene associated with susceptibility to benign tumours only (schwannomas), and is thus not included in table 1. The typical 1.8 Mb 22q11.2 distal deletion (DECIPHER GRCh37, 22: 21 917 117–23 722 445) does not include any CSGs, and so also has not been included in table 1. Only infrequent so-called type III 22q11.2 distal deletions include the *SMARCB1* gene, which is associated with elevation of risk of malignant rhabdoid tumour predisposition syndrome (MIM 609322).[39 40]

Defining types of CSG-SASIs

For our recommendations, we categorised CSG-SASIs into four groups, with regard to recommendations for clinical management (table 2).

Group 1

'Recognised' (micro)deletion/duplication syndrome involving a CSG for which there is a demonstrable elevated lifetime risk of cancer evident from the literature; (1a) high risk of cancer for which surveillance is recommended, (1b) elevated risk of cancer but surveillance not routinely recommended.

Group 2

'Recognised' (micro)deletion/duplication syndrome involving a CSG; no evidence of increased risk of the cancer(s) in question evident from the literature.

Group 3

Other (non-recognised) SA which involves a CSG which is of 'high-actionability' (HA-CSG); (3a) deletion or within-gene duplication (3b) large duplication.

Group 4

Other (non-recognised) SA which involves a CSG that is not a HA-CSG. Group 4 SAs were further subclassified according to; (i) whether they were a primary SA, a SA of uncertain significance (SAUS) or a non-primary SA, (ii) whether the CSG involved acts via LOF or GOF and (iii) the mode of inheritance (iv) type of SA deletion/within-gene duplication or large duplication. Four groups were delineated: (4a) primary SA/SAUS: deletion or within-gene duplication in a primary SA/SAUS for a gene which acts via LOF and is of autosomal dominant inheritance (4b) primary SA/SAUS : large duplication and/or gene acting by GOF and/or gene acting recessively, (4c) non-primary SA: deletions or within-gene duplication, (4d) non- primary SA: large duplication .

(3) Frequency of CSG-SASIs in literature-reported CMA series

From review of the literature, two publications, totalling 18 625 individuals, were identified comprising large retrospective case series in whom CMA was undertaken for clinical investigation of phenotypes largely unrelated to cancer[41 42] (see online supplementary file 2). Pichert *et al*[41] analysed CMA data from 4805

Table 1 Summary of 11 DECIPHER-recognised (micro) deletion/duplication syndromes involving a cancer susceptibility gene (Group 1 and Group 2 CSG-SASIs)

SA-related syndrome	DECIPHER coordinates, (GRCh37)	Typical size, range of SA	CSG involved (and associated cancer syndrome)	Association of SA-related syndrome with relevant cancer (literature)	SASI group
Cri du chat syndrome (5 p deletion)	5:10 001–12533304	12.5 Mb range 10–45 Mb	*SDHA* (paragangliomas 5, MIM 614165) *TERT* (acute myeloid leukaemia MIM 601626, melanoma, MIM 615134)	No	2
Familial adenomatous polyposis (micro)deletion syndrome	5:112043201–112181936	140 kb variable range	*APC* (familial adenomatous polyposis, MIM 175100)	Yes	1a
Sotos syndrome	5:175724636–177052116	1.3–2 Mb range 480 kb to 5 Mb	*NSD1* (Sotos syndrome including teratomas/neuroblastoma, MIM 117550)	Yes	1b
WAGR 11p13 deletion syndrome	11:31806339–32457087	651 kb range 1–26.5 Mb	*WT1* (Wilms' tumour, MIM 194070)	Yes	1a
Potocki-Shaffer syndrome	11:43994800–46052450	2.06 Mb variable size, at least 2.1 Mb	*EXT2* (multiple exostoses 2 and chondrosarcoma, MIM 133701)	No	2
16p11.2-p12.2 microduplication syndrome	16:21475060–29284077	7.81 Mb range 6.71–8.95 Mb	*PALB2* (familial breast cancer, MIM 114480)	No	2
16 p11.2-p12.2 microdeletion syndrome	16:21512062–30199854	8.69 Mb range 7.1–8.7 Mb	*PALB2* (familial breast cancer, MIM 114480)	No	2
Smith-Magenis syndrome	17:16773072–20222149	3.45 Mb range 1.5–9 Mb	*FLCN (*Birt-Hogg-Dube syndrome, MIM 135150)	No	2
NF1-microdeletion syndrome	17:29107097–30263321	1.16 Mb range 1.2–1.4 Mb	*NF1 (*neurofibromatosis type 1, MIM 162200)	Yes	1b
Potocki-Lupski syndrome (17p11.2 duplication syndrome)	17:16773072–2022149	range 3.45–3.7 Mb	*FLCN (*Birt-Hogg-Dube syndrome, MIM 135150)	No	2
Xp11.22-p11.23 microduplication syndrome	X:48334549–52117661	3.78 Mb range 0.8–9.2 Mb	*WAS* (Wiskott-Aldrich syndrome, MIM 301000), lymphoma	No	2

CSG, cancer susceptibility gene; SA, structural aberration; SASI, structural aberrations with secondary implications.

Table 2 CSG-SASI Groups 1–4: clinical-laboratory reporting and management recommendations

Group 1: recognised (micro)deletion syndrome involving a CSG; demonstrable increased lifetime cancer risk	**Group 2: recognised (micro)deletion/duplication syndrome involving a CSG; no demonstrable elevation of incidence of cancer**
Four recognised (micro)deletion syndromes involving a CSG. There is published evidence supporting an increased lifetime risk of cancer (table 1): a. High risk of cancer; surveillance recommended. b. Elevated risk of cancer; surveillance not routinely recommended.	Seven recognised (micro)deletion/duplication syndromes involving a CSG. No published evidence supporting an increased lifetime risk of cancer (table 1):
► CSG should be named clearly in the report, in both the prenatal and postnatal settings. ► Increased risk of cancer should be clearly stated. ► A proband with Group 1 SASI should be managed for the associated increased malignancy risk as per standard recommendations for the respective syndrome, namely: – 1a (*WT1*, *APC*): active surveillance. – 1b (*NF1*, *NSD1*): symptom awareness. ► Reflex parental testing – Can be performed for SASIs involving *NSD1*, *WT1* and *NF1*. – Should be preceded by genetics consultation for SASIs involving *APC*.	► CSG should be named clearly in the report, in both the prenatal and postnatal settings. ► Recognised association with risk of cancer of *pathogenic sequence variants in the gene* should be clearly stated. ► Absence of evidence for association with cancer for *(micro)deletion/duplications involving the gene* should be clearly stated. ► A proband with Group 2 SASI should not be managed for increased malignancy risk ► Reflex parental testing can be performed.
Group 3: other (non-recognised) SA involving a high-actionability CSG (HA-CSG)	**Group 4: other (non-recognised) SA involving a CSG which is not of high-actionability**
SASIs which include one of the 23 HA-CSGs a. Deletion or within-gene duplication. b. Large duplication encompassing the entire gene.	SASIs which involve a cancer susceptibility gene whichis not one of the 23 HA-CSGs. a. primary SA/SAUS: LOF, autosomal dominant (AD) gene: Deletion or within-gene duplication. b. primary SA /SAUS: Large duplication and/or gene acts by GOF and/or gene acts recessively. c. non-primary SA: Deletion or within-gene duplication d. non-primary SA: Large duplication
► CSG should be named on the report whether it is located in a primary SA, a SAUS or a non-primary SA, whether ascertained in the prenatal or postnatal setting, and whether a deletion or duplication (3a or 3b). ► Recognised association with risk of cancer of *pathogenic sequence variants in the gene* should be clearly stated. ► For 3a: it should be stated that an increased risk of cancer is anticipated, but it is unclear whether the risk is equivalent to that conferred by pathogenic sequence variants. ► For 3b: it should be stated that no increased risk of cancer is anticpated. ► A proband with Group 3a SASI should be managed for the associated increased malignancy risk as per the standard recommendations for the respective syndrome, at age indicated in figure 1. ► A proband with Group 3b SASI should not be managed for increased malignancy risk. ► Reflex parental testing – Can be performed for all 3b SASIs and 3a SASIs involving *RB1* and *WT1*. – Should be preceded by genetics consultation for other 3a SASIs	► Where the SA is a primary SA or SAUS, the CSG should be included on the report, whether ascertained in the prenatal or postnatal setting, and whether a deletion or duplication and regardless of gene mechanism of inheritance (4a, 4b). ► Recognised association with risk of cancer of *pathogenic sequence variants in the gene* should be clearly stated. ► For 4a: it should be stated that an increased risk of cancer is possible, but the magnitude of risk is unclear and unlikely to be equivalent to that of a pathogenic sequence variant. ► For 4b: it should be stated that no increased risk of cancer is anticpated . ► For a proband with Group 4a SASI, management of cancer risk should likely be modified down from standard recommendations for the respective syndrome and should be individualised according to the prognosis of the primary diagnosis and associated morbidities. ► A proband with 4b SASI should not be managed for increased malignancy risk. ► Reflex parental testing can be performed. ► Where the SA is non-primary (4c, 4d), neither the SA nor the CSG should be mentioned on the report.

CSG, cancer susceptibility gene; GOF, gain of function; HA-CSG, high-actionability CSG; LOF, loss of function; SA, structural aberration; SASI, structural aberrations with secondary implications.

individuals for involvement of 47 CSGs (of which, for the purposes of analysing 'secondary' SAs, we excluded 3 individuals referred with clear clinical syndromes involving CSGs—1 with Lynch syndrome, 2 with features consistent with NF1). Innes *et al*[42] examined CMA data from 3366 patients for 39 CSGs (pilot series) and 10 454 patients for 105 CSGs (extended series).

Pichert *et al*[41] identified 26 CSG-SASIs involving 13 different CSGs in 26/4802 individuals (0.54%). Innes *et al*[42] identified SASIs involving one or more CSGs in 31/3366 individuals in the pilot series (0.92%) and in 49/10 454 (0.47%) individuals in the extended series. Therefore, across a total of 18 622 individuals analysed, there were CSG-SASIs identified in 106/18 622 individuals (0.6%), comprising 119 instances of involvement of 40 different CSGs (12 of the SASIs involved 2 or more CSGs) of which 77 pertained to deletions and 42 to duplications (see online supplementary file 2).

Of the 119 instances of CSG involvement in the 106 CSG-SASIs, 34/119 (28.6%) occurred as part of recognised (micro)deletion/duplication syndromes, of which there was 1 Group 1a CSG-SASI (1 deletion in *APC*), 4 Group 1b CSG-SASIs (1 deletion of *NF1,* 3 deletions of *NSD1*) and 29 involved in Group 2 CSG-SASIs (28/119; 23.7%) involving *FLCN* (11 deletions, 3 duplications) and 7 deletions involving both *SDHA* and *TERT*. Thirty-seven of 119 (31.1%) were HA-CSGs involved in Group 3 CSG-SASIs comprising 16 instances of deletion and 21 of duplication and involved 12 of the 'ACMG 23 gene set' (*BMPR1A* (8), *BRCA2* (1), *MSH2* (1), *MSH6* (2), *PMS2* (4), *PTEN* (2), *RB1* (4), *SDHB* (1) *SDHD* (3), *TP53* (4), *TSC2* (5), *VHL* (2)). The remaining 48 instances of CSG involvement (48/119; 40.3%) were classified as Group 4, that is, neither part of a recognised (micro)deletion/duplication syndrome nor involving a 'high-actionability' CSG. These comprised 31 deletions and 17 duplications: *BLM* (1), *BRIP1* (1), *CDKN1B* (1), *CHEK2* (2), *FANCI* (1), *FH* (2), *GPC* (1), *HRAS* (1), *JAG1* (2), *KIT* (1), *MAX* (1), *MET* (4), *NF1* (1), *PDGFRA* (2), *PMS1* (1), *POLD1* (1), *POLE* (1), *PRKAR1A* (2), *PTCH1* (2), *RUNX1* (1), *SDHA* (2), *SMARCA4* (1), *SMARCB1* (10), *TERT* (1) and *TMEM127* (5).

Innes *et al*[42] further delineated in their study whether the SASI identified was felt to explain the clinical features for which the patient was being investigated (primary SASI), was an SA of uncertain significance or whether it was a non-primary SA deemed unrelated to the primary presentation. Of the 93 instances of CSG involvement in the 80 CSG-SASIs identified by Innes *et al*,[42] 67 were involved in primary SASIs, 10 in non-primary SASIs and 16 in SASIs of uncertain significance.

(4) Evidence for cancer penetrance of CSG-SASIs

For 22 of the 'ACMG 23 gene set', deletions involving the entire gene (with or without complex rearrangements) of a size typically detectable by CMA, have been reported on clinical investigation of cases with the associated cancer predisposition syndrome (exception *SDHAF2*).[3]

With regard to *retrospective evidence* regarding penetrance for cancer, for 19/80 and 4/26 of the CSG-SASIs identified in Innes *et al*[42] and Pichert *et al*,[41] respectively, the SASI was demonstrated to be inherited and family history information was available. In none of these 23 cases was there any reported family history of relevant cancers.[41] With regard to *prospective evidence,* Innes *et al*[42] reported two de novo RB1 deletions; both individuals are reported to have developed retinoblastoma subsequent to ascertainment of the deletion. One *TSC2* deletion was shown to be de novo and the child subsequently developed features consistent with tuberous sclerosis.[42] Otherwise, minimal further *prospective evidence* is available regarding cancer risk in individuals in whom a CSG-SASI was ascertained from these two studies, or more widely in the medical literature.

Recommendations

By categorising the SASIs into four discrete groups, our working group has generated consensus clinical-laboratory management recommendations, regarding (1) Reporting of findings in the context of a primary CSG-SASI or a non-primary-CSG-SASI. (2) How the associated cancer risk should be reported. (3) Reflex parental testing and pretest genetic counselling. (4) Management of potential associated cancer risk (table 2, figure 1).

In the UK, it is standard to include on the clinical report, along with its genomic coordinates, a primary SA (one deemed causative of the phenotype) or a SA of uncertain but putative clinical significance .[43] It would be possible therefore using publically available genome browser tools, for any clinician or parent to interrogate a SA delineated on a report for the genes contained therein. We therefore recommend that for any SA to be included of the report, its is routinely interrogated for all CSGs and any CSG found to be involved is mentioned along with a clear statement regarding likelihood of increased risk of cancer and recommendations regarding clinical management. A SA deemed non-primary (non-causative of the presenting feature(s) under investigation) would not typically be mentioned on the report.

We recommend that all SAs (primary, uncertain and non-primary) are *routinely* interrogated for the inclusion of HA-CSGs and if an HA-CSG-SASI is detected, this should be included on the report regardless (Group 3 SASIs).

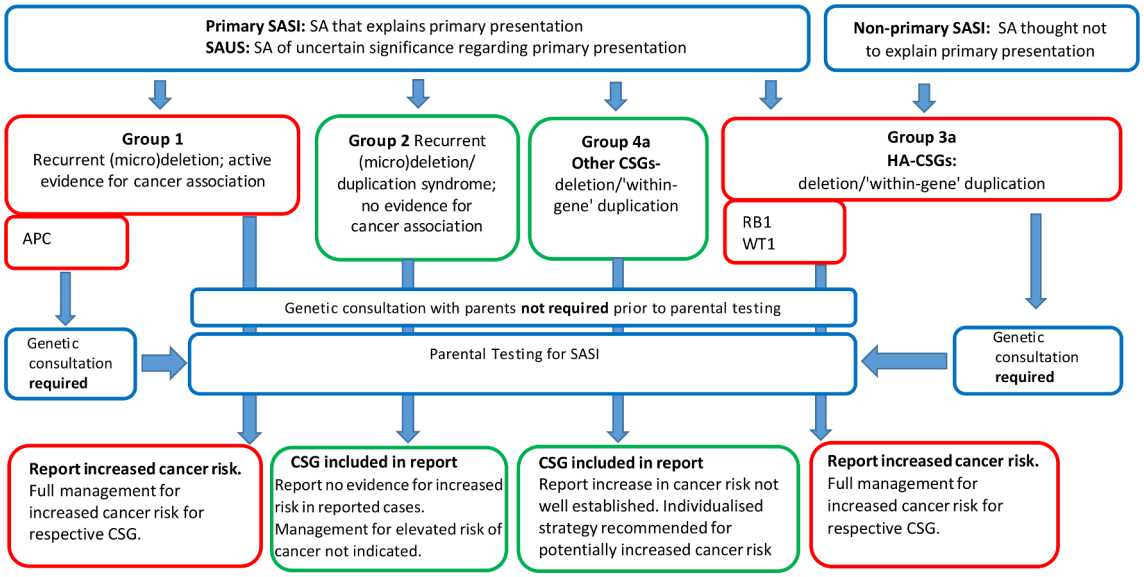
CSGs which are not of high actionability should not be *routinely* interrogated for. CSGs not of high actionability will therefore only be mentioned on the report when included in a primary SA or an SA of uncertain significance (SAUS). For large duplications (which fully encompass the gene), the involved CSG should be mentioned in the report but the report should state that increased cancer risk is not anticipated and no additional management for elevated cancer risk is recommended (Group 3b and Group 4b CSG-SASIs).

We do not recommend active management for cancer risk for those well-recognised (micro)deletion/duplication syndromes for which no increased incidence has been reported across the literature of the cancer associated with the CSG (Group 2 SASIs).

Regarding parental reflex testing, this only need be delayed in selected scenarios for which there is a significant likelihood of providing unsolicited prediction of future cancer risk for the parents, namely (1) For Group 3a CSG-SASIs for which the associated cancers are of late onset. (2) For *APC*-containing deletions (Group 1a CSG-SASI). Involvement of a clinical geneticist with expertise in cancer susceptibility will be required to define appropriate management strategies following detection of a Group 1, Group 3a or Group 4a CSG-SASI. In all cases, the overall prognosis and associated morbidities of the patient should be taken into account when planning the clinical management.

DISCUSSION

The apparent lack of cancer association for many CSG-SASIs, for example, the recognised CSG-SASIs comprising Group 2, may reflect the impact of different molecular mechanisms between pathogenic sequence variants and SAs. This difference may be mediated by involvment within SAs of *cis* regulatory elements which influence expression of the gene. Of the 11 recognised (micro)deletion/duplication syndromes containing CSGs, the



Group 3 High Actionability Cancer Susceptibility Genes (HA-CSGs): Earliest penetrance for cancer typically in:								
Early childhood			Late childhood (10+)			Adult (18+)		
RB1 TP53	TSC1 TSC2	VHL WT1	APC MEN1 NF2	SDHD SDHAF2 SDHC PTEN	SDHB STK11 BMPR1A SMAD4	BRCA1 BRCA2	MLH1 MSH2	MSH6 PMS2

Figure 1 Workflow management for CSG-SASIs. Including indication for genetic counselling ahead of parental testing, follow-up for increased cancer risk and initiation age for management of risk. CSG, cancer susceptibility gene; HA-CSG, high-actionability cancer susceptibility gene; SA, structural aberration; SASI, structural aberration with secondary implication.

2 for which penetrance of cancer is high (*APC* and *WT1*) are those for which the region of deletion is typically smallest (<0.7 Mb). In further support of this hypothesis, it has been demonstrated for that larger deletions of *VHL* on chromosome 3p25.3 involving contiguous loss of C3orf10 are associated with lower risk of renal cancer.[44]

Alternatively, the apparent absence of penetrance for cancer may purely be a function of ascertainment agnostic to cancer phenotype and unrelated to molecular mechanism. This hypothesis would be in fitting the well-established high penetrance of sequence variants in *APC* and *WT1*, meaning that the cancers are still penetrant in individuals with SAs regardless of the context of ascertainment. Another plausible contributory factor may be underascertainment of cancer in probands with complex syndromic intellectual disability, on account of (1) Impaired overall survival and/or (2) Less aggressive medical investigation.

Intragenic ('within-gene') duplications that disrupt the coding region of a gene and result in nonsense-mediated decay are widely accepted as causing LOF. For example, a 2631 base pair duplication in *SMARCB1* (MIM 162091/609322) has been reported as causing schwannomatosis and rhabdoid tumours.[45] In current practice, fine-mapping data have seldom been available to accurately define the break points of intragenic duplications; increasing availability of long-read technologies may improve the precision with which such duplications are delineated. Impact is uncertain for the more common 'large duplications' which fully encompass and do not disrupt the coding region. Similarly uncertain is the effect of deletions or duplications on GOF CSGs, although there have been isolated reports of association, for example, of germline deletions in *RET* with risk of glioblastoma multiforme.[46]

In the case of well-recognised SA-related syndromes for which there is no association with cancers reported in the literature, despite hundreds of reported cases , we do not recommend active management in the context of unproven cancer risk (Group 2 SASIs). As Group 4a SASIs are non-recurrent, there are little data with which to infer penetrance or inform management recommendations. Empirically, the cancer risk is likely to be proportional to the underlying penetrance for a typical LOF pathogenic variant in the gene (and possibly inversely related to the size of the SA). For these Group 4a SASIs, pragmatic individualised approaches will be required, based on typical penetrance of the gene, the availability and acceptability of interventions, patient-specific prognosis and concurrent morbidities.

Unbiased national longitudinal data linkage are urgently required to better quantify cancer outcomes for individuals with SASIs so as to generate improved prospective estimates of risk. Data governance issues notwithstanding, linkage of individual-level data is entirely feasible via alignment of molecular data to routinely collected national registries of cancer incidence.

The rapid roll-out of clinical WGS, particularly within the UK National Health Service, offers additional challenges regarding reporting of SASIs. The improved sensitivity of WGS for small SAs is accompanied by poorer specificity and positive predictive value (precision). Furthermore, WGS has the potential to reveal copy-number neutral SAs not previously detectable on CMA, about which very little data currently exist.

SUMMARY

From our analysis of 18 622 CMA results from individuals referred for phenotypes unrelated to cancer, approximately 0.6% individuals (106/18 622 or 1/175) had a CSG-SASI detected. Of the 119 instances of CSG involvement, 4.7% were Group 1, 19.8% were Group 2, 33.0% were Group 3 and 42.5% were Group 4 CSG-SASIs.

Via our recommendations, clinical management for elevated cancer risk would have been indicated for, at most, 40 cases,

comprising 5 Group 1 CSG-SASIs (1 deletion in *APC*, 1 deletion of *NF1*, 3 deletions of *NSD1*), 17 Group 3a CSG-SASIs (16 deletions and 1 partial duplication of *TSC2*) and 18 Group 4a SASIs (primary, uncertain or unknown SAs, involving deletion of a LOF autosomal dominant gene), equating in total to 0.23% of all CMA subjects (40/18 622). The majority of these are primary SASIs and de novo, so familial cascading for the CSG-SASI would therefore be highly infrequent. In these guidelines, we have sought to (1) Reduce the potential delays in laboratory processing when a SASI is identified. (2) Improve consistency in information provided when a SASI is reported and how patients are managed. (3) Balance the benefits of cancer prevention/early detection against the psychological, social and economic harms of lifelong monitoring where a theoretical risk of cancer is poorly substantiated.

It remains imperative to provide appropriate expert information and counselling for patients and families undergoing genetic analysis, including communication of uncertainty, alongside continued education to relevant non-genetic professionals. Due to the current paucity of longitudinal data, ambiguity regarding cancer risks will likely persist for many decades. Improved data linkage is urgently required to empower robust data on longer-term outcomes.

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