Genetics inMedicine | CORRESPONDENCE

Response to Gorokhova et al.

In our paper "The ARID1B spectrum in 143 patients: from nonsyndromic intellectual disability to Coffin–Siris syndrome"[1] we compared the phenotypes of 79 patients with an a priori clinical diagnosis of Coffin–Siris syndrome (CSS) and a pathogenic variant in *ARID1B*, and 64 patients where the causal variant in *ARID1B* was identified through genome-wide techniques without a clinical suspicion.

In their correspondence Gorokhova et al.[2] point out the significant role of intergenic deletions in the mutational spectrum of *ARID1B*. They describe 45 patients with pathogenic variants in *ARID1B*, collected via several diagnostic laboratories, including 6 intragenic deletions (13%). Three deletions were identified through gene panel sequencing including copy-number variant (CNV) analysis, two were initially missed via this method but picked up on targeted reanalysis, and one was identified through multiplex ligation-dependent probe amplification (MLPA).

In our cohort of 143 patients with pathogenic variants in *ARID1B*,[1] 6 patients had whole-gene deletions, and a further 12 had intragenic deletions of *ARID1B*. All whole-gene deletions and 10/12 intragenic deletions were identified via chromosomal microarray (CMA); the remaining two (patients 31 and 134) were identified via MLPA. Because screening for intragenic deletions was not systematically performed in our patients, our data do not lend themselves to determining the contribution of intragenic variants. However, we previously published a CSS cohort of 63 patients,[3] which is to our knowledge still the only cohort where *ARID1B* was systematically screened for intragenic deletions using MLPA. In this cohort we found only a single intragenic deletion (exons 6–9, patient 31 in the current cohort), one whole-gene deletion, and 26 other truncating variants. Based on this, we previously recommended that smaller CNVs should be investigated by MLPA when *ARID1B* is considered a likely causal gene,[4] but the data presented by Gorokhova and colleagues suggest that CNV analysis on gene panel data can also be sensitive.

Since linking *ARID1B* to Coffin–Siris syndrome[5] we have offered *ARID1B* sequencing (first by Sanger sequencing, later by targeted next-generation sequencing [NGS]) and MLPA as a diagnostic test. Including only those requests that involved screening of the whole gene (thus excluding confirmations of research findings, e.g., exome sequencing [ES] studies), we detected 23 pathogenic variants in *ARID1B*, two of which were intragenic deletions (exons 6–10; 13). In total therefore, we detected three intragenic deletions in 51 patients (6%), of which two are detectable by high-resolution CMA.

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We do not know why our number (6%) differs from the number quoted by Gorokhova et al.[2] (13%). This may be due to random variation, or, as previously discussed,[3] it is possible that our cohort had fewer intragenic deletions because these were previously picked up by CMA, and therefore not sent to us for analysis. On the other hand, the cohort presented by Gorokhova and others may somehow be biased toward patients with small deletions, possibly due to selection bias, but alternatively for biological reasons. For *NSD1* for example, it has been suggested that the contribution of intragenic deletion differs between Japanese and non-Japanese populations,[6] although we suspect that the majority of patients in the two *ARID1B* cohorts are of Caucasian ancestry. Regardless of the cause, it is clear that intragenic pathogenic variants in *ARID1B* are not rare.

So how can these be identified? When the clinical suspicion is high, MLPA or focused CNV calling on NGS data can be performed. If in spite of sufficient analysis for intragenic deletions no variant is found and clinical suspicion remains high, karyotyping can be considered since there have been four reports of translocations through *ARID1B*. Another intriguing possibility is to investigate the epi-signature in blood. Recently, BAFopathies including *ARID1B*-related intellectual disability were shown to have a specific epi-signature.[7] Thus, in cases with a high clinical suspicion one could consider performing a DNA methylation test. When the profile is specific for BAF complex involvement intragenic variants could be more precisely investigated, for example by genome sequencing.

However, as we have shown, there are many patients with pathogenic variants in *ARID1B* that do not have specific features,[1] and therefore only investigations using genome-wide techniques would be offered to these patients. In our population, many intragenic deletions (exons 1–5, 5–6, 6, 6–7, 6–8) were identified by CMA, which partially overlap with cases 1, 2, 4, and 6 presented by Gorokhova et al.[2] It is clear that the resolution of CMA differs significantly between diagnostic laboratories. Although Gorokhova and colleagues point out that several arrays suffer from a lack of probes in exons 9–20, many diagnostic labs use higher-resolution arrays. For example, our diagnostic laboratory has used the Agilent Cytoscan HD array, which has over 50 probes in this region, since 2011.

These days, most patients with intellectual disability (ID) also undergo ES or gene panel analysis. CNV calling on such data is possible, but to balance sensitivity and specificity it is usually restricted to calls in multiple adjacent exons.[8] We propose to increase sensitivity by decreasing such thresholds for genes that are frequent causes of ID and have haploinsufficiency as the main mechanism, such as *ARID1B* and *ANKRD11*.

In conclusion, we agree with Gorokhova and colleagues that intragenic deletions in *ARID1B* are a relevant part of the mutational spectrum. Interestingly, we have observed a sharp decrease in diagnostic requests for *ARID1B* in recent

years, although we are the only diagnostic laboratory offering MLPA for *ARID1B* in the Netherlands. In our experience, most geneticists will request trio ES at their local laboratory when CSS is suspected given the high number of genes involved. Intragenic deletions may thus be missed especially in the absence of sensitive CNV-calling on ES data. We highly recommend screening for intragenic deletions of *ARID1B* in patients with a clinical suspicion of CSS, and further propose to increase CNV detection sensitivity for frequent ID genes such as *ARID1B* by decreasing stringency for these genes when calling CNVs on exome sequencing and panel data.

DISCLOSURE

The authors declare no conflicts of interest.

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