Multidisciplinary interaction and MCD gene discovery. The perspective of the clinical geneticist

Grazia M.S. Mancini [a, g, *], Daphne J. Smits [a, 1], Jordy Dekker [a, 1], Rachel Schot [a, g], Marie Claire Y. de Wit [b, g], Maarten H. Lequin [c], Marjolein Dremmen [d, g], Alice S. Brooks [a], Tjakko van Ham [a], Frans W. Verheijen [a, g], Maarten Fornerod [e], William B. Dobyns [f], Martina Wilke [a, g]

[a] Department of Clinical Genetics, ErasmusMC University Medical Center Rotterdam, Dr. Molewaterplein 40, 3015 GD, Rotterdam, the Netherlands
[b] Department of Child Neurology, Sophia Children's Hospital, ErasmusMC University Medical Center Rotterdam, Dr. Molewaterplein 40, 3015 GD, Rotterdam, Rotterdam, NL, the Netherlands
[c] Department of Radiology, University Medical Center Utrecht, Utrecht, the Netherlands
[d] Department of Radiology, Sophia Children's Hospital, ErasmusMC University Medical Center Rotterdam, Dr. Molewaterplein 40, 3015 GD, Rotterdam, the Netherlands
[e] Department of Cell Biology, ErasmusMC University Medical Center Rotterdam, Dr. Molewaterplein 40, 3015 GD, Rotterdam, the Netherlands
[f] Department of Pediatrics (Genetics), University of Minnesota, 420 Delaware Street SE, MMC75, Minneapolis, MN, 55454, USA
[g] ENCORE Expertise Center for Genetic Neurocognitive Developmental Disorders, Erasmus, MC, Rotterdam

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ABSTRACT

The increasing pace of gene discovery in the last decade has brought a major change in the way the genetic causes of brain malformations are being diagnosed. Unbiased genomic screening has gained the first place in the diagnostic protocol of a child with congenital (brain) anomalies and the detected variants are matched with the phenotypic presentation afterwards. This process is defined as "reverse phenotyping". Screening of DNA, through copy number variant analysis of microarrays and analysis of exome data on different platforms, obtained from the index patient and both parents has become a routine approach in many centers worldwide. Clinicians are used to multidisciplinary team interaction in patient care and disease management and this explains why the majority of research that has led to the discovery of new genetic disorders nowadays proceeds from clinical observations to genomic analysis and to data exchange facilitated by open access sharing databases. However, the relevance of multidisciplinary team interaction has not been object of systematic research in the field of brain malformations. This review will illustrate some examples of how diagnostically driven questions through multidisciplinary interaction, among clinical and preclinical disciplines, can be successful in the discovery of new genes related to brain malformations. The first example illustrates the setting of interaction among neurologists, geneticists and neuro-radiologists. The second illustrates the importance of interaction among clinical dysmorphologists for pattern recognition of syndromes with multiple congenital anomalies. The third example shows how fruitful it can be to step out of the "clinical comfort zone", and interact with basic scientists in applying emerging technologies to solve the diagnostic puzzles.

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Contents

1. Introduction

The etiological diagnosis of brain malformations is a complex and time-consuming process that demands skills and collaboration with different specialists and with the patient family. It has been clearly established that the recognition of a known cause has important implications for disease management and family counseling [1,2]. Brain malformations, especially malformations of cortical development (MCD) are extremely variable in terms of morphological abnormalities, pathogenic mechanism, affected areas of the brain, hence clinical manifestations [3,4]. Among the major consequences of MCD are intellectual disability (ID), epilepsy including practically all types of epileptic syndromes, motor impairment and any sort of movement disorders, behavioral issues like autism spectrum disorders and more. However, in the less extensive forms, MCD can go unnoticed or cause only borderline behavioral or cognitive issues, not reaching the need for a full diagnostic work-up and may be discovered after a sporadic seizure event or after genetic diagnosis in a family member [5,6].

After the advent of magnetic resonance imaging (MRI), MCD were classified according to distinct imaging patterns [7]. During past three decades, the contribution of monogenetic disorders to the etiological constellation of MCD has been increasingly recognized and has led to the establishment of morphological and genetic —based correlations [3]. These, in turn, have been instrumental to direct and speed up the diagnostic workup in the daily practice. While data on the timeline of the diagnostic trajectory for MCD are scarce, if not absent, data derived from diagnostic follow-up of related pathologies such as intellectual disability (ID) and neurodevelopmental disorders (NDD), show that the "odyssey" usually covers years of investigation, with obvious financial and psychological burdens to families and society [8].

The introduction of next generation sequencing about two decades ago has strongly facilitated the discovery of pathogenic variants in genes involved in brain development, shedding light on an unexpected huge genetic heterogeneity and pleiotropy within NDD and MCD. On the one hand, this heterogeneity has made it increasingly difficult to correlate the clinical and imaging presentation to a specific genetic cause. On the other hand, the wide genetic heterogeneity has led to widespread implementation of genome-based screening tests, like whole exome sequencing (WES), as diagnostic common practice.

The "genotype first" approach has been claimed and implemented with success as first tier test for the diagnosis of common neurological disorders like NDD, where more than 2000 different genes have been proven to be causally responsible [9]. This approach is much less straightforward than it may appear because of several issues inherent to the strategy. There are pitfalls related to the interpretation of variants of unknown significance (VUS). For example if multiple phenotypes are linked to the same gene [10,11]), or in case of incidental findings on unrelated disorders [12,13]. To complicate the story, several studies in extended population cohorts have shown that MCD are among the most common incidental findings at MRI of control childhood population. As Jansen PR et al. reported in 2017 [14], studying a cohort of 3966 healthy children and later confirmed in a much larger cohort of 11679 children, recently published by Li Y et al. in JAMA [15], periventricular nodular heterotopia and other MCD accounted for about 0,5-1% of all incidental findings. These data clearly indicate the need for appropriate genetic variant interpretation and phenotype correlation before a definitive diagnosis can be reached. This requires close collaboration within dedicated teams of clinical geneticists, laboratory geneticists, neurologists and neuroradiologists.

1.1. Phenotype match and syndrome description

The widespread application of exome sequencing in the diagnostic setting, but also the increasing amount of genes present in the panels, constantly leads to the identification of VUS in genes that appear to be excellent candidates to explain the phenotype. The availability of user friendly open access platforms, such as Genematcher (https://genematcher.org/ [64]) or LOVD (https://www.lovd.nl/3.0/home [65]), has facilitated exchange of information on novel variants. This gives the opportunity to clinicians and geneticists world-wide to compare patient phenotypes, augmenting phenotypic description of known syndromes. It also enables identification of genes related to sometimes extremely rare disorders, often primarily based on statistic evidence of phenotype-genotype association for monogenetic (Mendelian) traits [16,17]). Nonetheless, the description of new genetic disorders is not always accompanied by thorough phenotyping, often because each syndrome is very rare [17,18]. A recent review has identified inaccurate phenotyping as a major drawback in the definition of novel syndromes. This has led to a consensus statement by a group of renowned geneticists, pediatricians and scientists, who suggest for the taxonomy of rare genetic disorders a "dyadic approach to the delineation of diagnostic entities", including 1) evidence for pathogenic gene variant associated with 2) one or more, as distinct as possible, phenotypes [19]. No need to say that such an effort requires a multidisciplinary collaboration.

Here we illustrate our experience with the recent discovery of new MCD-associated syndromes as examples of how relevant and fruitful it is, in this process, to work in a multidisciplinary setting in order to pursue 1) accurate imaging phenotyping, 2) detailed dysmorphologic description and 3) utilization of advanced techniques for genomic analysis. This paper is a summary of the presentation at the satellite meeting organized by the Neuro-MIG network (COST Action CA16118) during the 13th European Pediatric Neurology Conference held in Athens on September 17th, 2019.

2. Results/examples

1 Interaction among neurologists, laboratory and clinical geneticists and neuroradiologists and the discovery of the MACF1-associated MCD.

In the fall of 2017 during one of the MRI review sessions organized by one of the authors (WBD) we discussed a case of a boy with an unusual MCD, where open trio exome sequencing, performed in diagnostic setting, detected a *de novo* variant in the GAR domain of the *MACF1* gene ((Ensembl: ENST00000372915.7 (MACF1-204) transcript; p.Cys7135Phe [66]). The MRI scan showed a diffuse moderate lissencephaly with a mild P > A gradient, hypoplasia of the anterior commissure, hypoplasia and dysplasia of the brainstem and particularly the pons, with defective midline pons decussation, most evident at DTI, indentation of the ventral pons on axial images, laterally placed and uncrossed pyramids [20] in Ref. [20]. Because this pattern seemed very distinctive and unusual, the databases of the participants were screened for these radiological features. At that time the literature report associating a defect in the decussation of the pontine bundles with a cortical malformation, by Ref. [21] showed an MRI pattern reminiscent of this initial patient with the *MACF1* variant. Based on the MRI appearance, a total of 8 patients, including the patient from Irahara et al. were selected from the databases of the session participants and were tested for variants in *MACF1*. MACF1, the microtubule-actin crosslinking factor 1, also known as ACF7, is a huge cytoskeletal protein with the function to cross link the subplasmalemmal actin cytoskeleton network to the microtubule network. Several isoforms of the mammalian MACF1 protein are known, some of which are brain-specific [22]. One of these is corresponding to the *MACF1*-204 transcript and fulfills a crucial role in extension of neuronal growth cone and neuronal migration (for review [23,24]). The microtubule binding function of MACF1 is mediated by the Zinc-binding at the GAR domain; four amino acids are involved in coordination of the Zn^{2+} ion [25]. Several animal studies have demonstrated that integrity of this domain is essential for normal brain development, while mono- or biallelic disruption leads to severe brain malformations in the mouse, in the worst case incompatible with life (for review see Ref. [26]. All but one of the tested patients showed a *de novo* missense variant leading to amino acid substitutions of one of the four GAR domain residues, previously known to be essential for coordination of Zn^{2+} ions. Interestingly, the patient reported by Irahara was the one not showing missense variants in the coding sequence of the gene. Additional (manual) sequence analysis revealed a small intragenic deletion of multiple exons (NM_012090.5 or exons 62—93 (p.Ala5498_Arg7150), using transcript MACF1-204), causing a deletion of two of several amino acids including the four amino acids needed for Zn^{++} binding in the GAR domain. These observations led to two main conclusions: 1) amino acid substitutions in one specific domain of the MACF1 protein are deleterious for the human brain and 2) they cause a recognizable pattern of brain malformation that warrants specific genomic analysis of this MACF1 domain in undiagnosed individuals [20]. In this respect, the MACF1-related brain malformation fulfills the criteria for a dyadic diagnostic entity. This conclusion seems even more relevant in view of several reports of MACF1-related entities, such as risk variants for schizophrenia [27—29], or bipolar disorders [30], or a neuromuscular disorder defined as spectraplakinopathy [31] or cases of fetal akinesia [32]. All these overt reports are limited to single observations or result from GWAS studies, hence do not fulfill the above mentioned diagnostic criteria. To a confirmation of the value of pattern recognition at brain imaging, a targeted test in a previously undiagnosed patient revealed the p.Asp7186Tyr

pathogenic variant substitution, leading to diagnostic confirmation [33]. This is one of the rare MCD disorders where the brain morphology can predict the genotype.

2 Interaction within a team of clinical geneticists and its relevance to identification of INTS1 and INTS8-related disorders

Dysmorphology has traditionally been a powerful instrument in the identification of genetic syndromes and still has a prominent place in the postgraduate training of specialists in medical genetics. However, the postgraduate training curriculum of other specialists dealing with genetic disorders, e.g. child neurologists, in the rule does not include a mandatory training period in dysmorphology. Besides several authoritative texts, many computer-based databases have been developed to support the diagnostic approach to the dysmorphic patient and their application has increasingly become user-friendly and is frequently used also by non-geneticists ([34]; https://www.face2gene.com/ [67]; https://cliniface.org/ [68]). Another major resource for syndrome classification are the common definitions for phenotypic description in the Human Phenotype Ontology (http://humanphenotype-ontology.github.io/. [69]).

In the process towards the identification of a new MCD syndrome, dysmorphology has been instrumental to lump and classify entities related to the same gene or pathway. Classical examples are the MCD related to Miller-Dieker syndrome, the first "contiguous gene" syndrome discovered in the early 80ies, pioneer of the many "microdeletion" syndromes discovered later, or the FLNA-related PNH [35,36]. For some, like Baraitser-Winter syndrome, the identification of pathogenic variants in the ACTB or ACTG1 gene has helped recognition of frontal-predominant lissencephaly as part of entities previously thought to be genetically distinct, e.g. Fryns-Aftimos syndrome and cerebrofrontofacial syndrome types 1 and 3 [37]. Also in the era of post-genomic phenotyping, recognition of similar dysmorphic features still has great relevance in the delineation of novel MCD entities. In the process of gene discovery, the recognition of undescribed dysmorphic phenotypes is not always supported by the available books or databases. Therefore, the multidisciplinary collaboration between (child)neurologists and clinical geneticists can be particularly efficient and fruitful, as illustrated by the following example.

In a sibship of four individuals, born to healthy and unrelated parents, two brothers and a sister presented with deep developmental delay, severe impairment of communication, motor skills and epilepsy. At MRI a similar pattern of periventricular nodular heterotopia prominent in the frontal areas and cerebellar vermis hypoplasia was detected in all three, which suggested a monogenetic cause of the MCD (Fig. 1) [38]. Mild dysmorphic features were noted in all three, consisting of short stature, broad high forehead, hypertelorism, prominent glabella, down slanting palpebral fissures, epicanthic folds, broad nasal tip, low-set dysplastic ears, downturned corners of the mouth, broad overlapping toes or fingers. While classic linkage analysis did not lead to a locus identification, whole genome sequencing in all family members identified compound heterozygous variants in the *INTS8* gene in all three affected subjects and heterozygosity in the fourth not affected and in both parents, compatible with autosomal recessive inheritance. INTS8 is a subunit of the Integrator complex, necessary for processing and maturation of major classes of small nuclear RNAs (U1/U2 snRNA), which in turn are major components of the spliceosome of most mRNA coding genes [39].

INTS8 is a large gene extremely intolerant to loss of function variants (pLI score 0.94) (https://gnomad.broadinstitute.org/ [70]), the detected variants are not described in any control cohort, which means that they are very rare also in heterozygosity, and in vitro
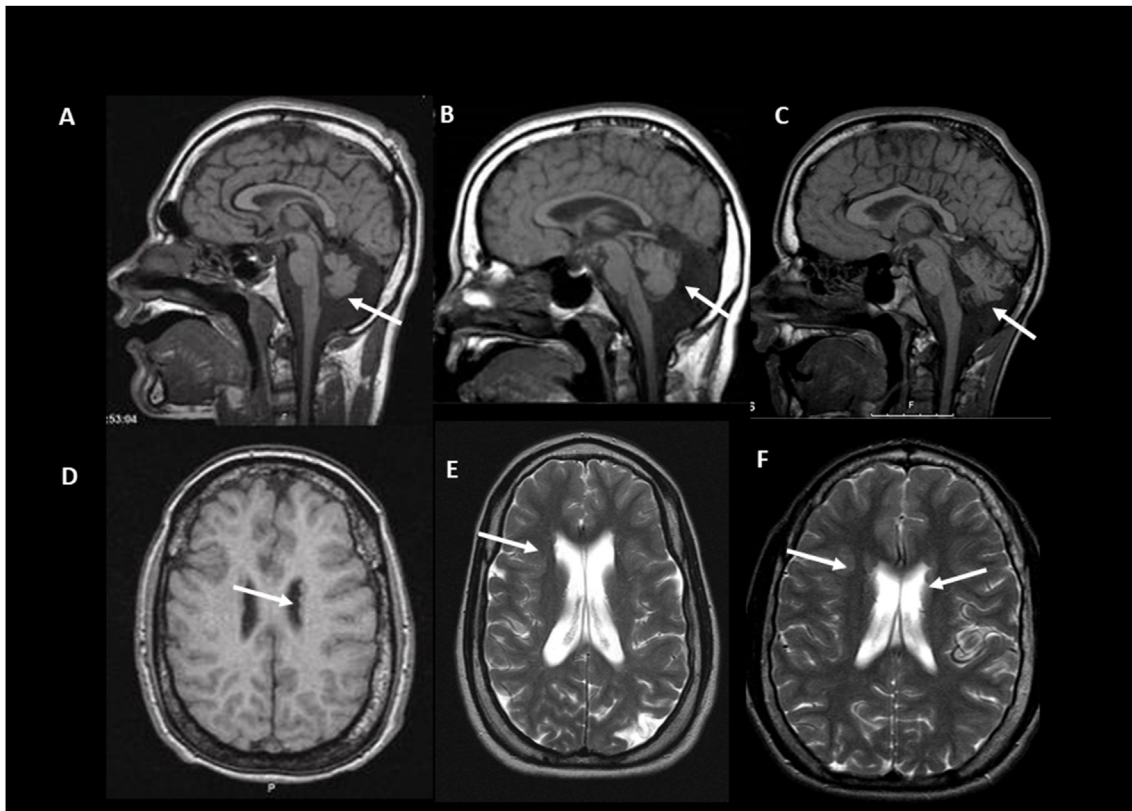
Fig. 1. Brain MRI images of three siblings affected by *INTS8* pathogenic variants and described by Oegema R et al. (2017). A,D: sibling 1; B,E: sibling 2; C,F: sibling 3. A,B,C midsagittal T1-weighted sections showing pronounced atrophy/hypoplasia of the cerebellar vermis (arrows) and mild atrophy of the corpus callosum. T1 (D) and T2 (E,F) -weighted axial images showing discrete periventricular nodular heterotopia (arrows). Mild dilatation of the lateral ventricles is present in E and F.

functional tests have shown loss of integrator complex integrity in the presence of the mutated protein [38]. All this evidence strongly imputes *INTS8* as the disease gene in the index family, however based on a single sibship it is still doubtful to use the variants as predictive genetic test. Unexpected support in favor of a causal relationship came from the description of three unrelated children with an overlapping dysmorphic phenotype, all sharing the same homozygous truncating pathogenic variant in *INTS1*, coding for another subunit of the integrator complex and interacting with INTS8. The dysmorphic features appeared so distinct and recognizable, that during presentation of the first case to the department team of clinical geneticists, two additional individuals were selected and tested by targeted DNA sequencing of *INTS1* based on their dysmorphism. Together, the similarity of the dysmorphic phenotype and functional relation between INTS1 and INTS8, led to the confirmation of a causal relationship between genotype and phenotype for both gene pathogenic variants and facilitated the identification of additional cases in short time [38,40].

3 Interaction with scientists and implementation of innovative techniques: identification of SMPD4 pathogenic variants

The introduction of exome sequencing has facilitated the identification a large number of MCD genes in the last decades. The department of Clinical Genetics of the ErasmusMC Academic Medical Center in Rotterdam has introduced since a decade diagnostic NGS panels for MCD. The first custom made panel version was based on targeted enrichment of about 103 genes and was run on the illumina MiSeq sequencing platform; the last version released in February 2021 is exome based and includes around 220

genes (https://www.erasmusmc.nl/nl-nl/patientenzorg/laboratoriumspecialismen/klinische-genetica [71]). Introduction of the exome-based panels is cost-effective, gives flexibility and allows inclusion or exclusion of genes on regular (in our case twice a year) basis. It allows testing of single patients without need for initial inclusion of the parents in the (trio) analysis, which makes the request accessible outside the setting of genetic counseling also to non-geneticists. WES-based panel analysis has also the advantage in limiting the detection of unsolicited findings. In the suspicion of a Mendelian disorder, it has been routine in the setting of genetic counseling at our Erasmus MC department, to offer a trio open (full) exome sequencing to panel-negative patients. This approach has been instrumental to the identification of new MCD genes, as illustrated above. However, this technology has nonetheless the limitation of covering with the exome only the coding sequences, i.e. circa 3% of the genome. The suspicion of a Mendelian trait inheritance in exome-negative cases can be based on phenotypic similarity of multiple affected individuals from the same (consanguineous) pedigree and evidence for linkage to a specific chromosome locus. This has been the case for our first family harboring pathogenic variants in the *SMPD4* gene [41]. This family has an extended consanguineous pedigree, including three sibships with four affected infants presenting with a severe infantile encephalopathy, congenital microcephaly with simplified gyral pattern and congenital arthrogryposis. Genomic linkage analysis identified a single locus on the long arm of chromosome 2, homozygous in all affected children. This region of autozygosity represented strong evidence for mapping of the disease gene to this chromosome area. Autozygosity mapping has been extremely important in MCD gene discovery in inbred populations, and particularly in the genetics of

primary microcephaly [42]. The lack of any significant coding variant in the circa 300 genes present in the homozygous area induced us to explore new techniques in collaboration with basic scientists.

High throughput RNA sequencing (RNA-seq) was chosen as a technique to investigate the transcriptome in the hope that a transcript abnormality, specifically derived from a non-coding DNA pathogenic variant, would be detected among the genes expressed from the linkage area. The RNA-seq technique has been widely explored for characterization of specific signatures in diseased tissues, mostly tumors or lymphoproliferative disorders [43], but only recently it received attention as tool complementing the diagnostic sequencing techniques, both at whole genome or at exome sequencing level for Mendelian disorders [44—48]. In order to detect the abnormal transcript by RNA-seq, it is important to carefully choose the tissue where the gene expression profile is being tested. Diagnostic applications have included diagnosis of muscular diseases using RNA isolated from muscle biopsies or from cultured myotubes derived from skin fibroblasts [45,46]). Recently, blood cells have also been used for the diagnosis of blood-unrelated disorders [47,48]. The RNA-seq dataset from patient cells can at the same time be used for analysis of differentially expressed genes and for pathway analysis, adding information on the gene function. Although not the ideal tissue, skin fibroblasts express many genes present in brain and neuronal lineages [44]; GTEx consortium, Nature 2017, doi:10.1038/nature24277 [49]; PAGE:, https://page.ccm.sickkids.ca [72]). As deduced from these databases, about 70% of the genes included in our WES-based panel is expressed in skin fibroblasts. Because of the availability of patient derived cells in these cases, we chose to compare gene expression profiles from controls and patient-derived skin fibroblasts and focused on the genes expressed from the linkage area on chromosome 2q22. In the linkage area only the expression of one gene, SMPD4, was significantly reduced, compared to controls. Reads of the residual SMPD4 transcript showed alternative sequences, either leading to frameshifts or to retention of intron 14. Retrospective analysis of the exome data showed a homozygous intronic variant: GRCh37 g.130912841C > T NM_017951.4 c.1407-9G > A p.? The variant was not called by the open exome analysis pipeline, which includes exon borders limited to +3 and −3 intronic sequence. After querying the Genematcher platform, through Neuro-MIG network and multidisciplinary collaborations, many additional cases were identified and confirmed a recognizable disease phenotype linked to recessive SMPD4 loss of function variants [41]. This confirmed a loss of function pathogenic SMPD4 variant as the cause of the disease segregating in our pedigree. SMPD4 is expressed both in fibroblasts and in the brain during human development. Additional proteomics analysis of SMPD4 interactors revealed that the SMPD4 protein is essential for ER and nuclear envelope membrane homeostasis, and thereby influences the mitotic process, which explains the proliferation defect causing congenital microcephaly in patients [41]; Smits D et al., manuscript in preparation).

3. Discussion

We have reviewed here three different approaches that have led to successful identification of new genes involved in MCD, each of them starting from observation of undiagnosed patient and involving the collaboration among several disciplines.

Multidisciplinary team management has increasingly been implemented in the care of complex pathologies, more commonly in the field of oncological disorders. Prospective studies in the field of cancer genomics have shown that a multidisciplinary genomics review board is needed not only for appropriate interpretation of

the genomic data obtained from tissue analysis, but also for stratification of patients for the appropriate therapy, for referral to clinical trials and for clinical genetics review [50]. Also, recommendations have been made for the organization of multidisciplinary care teams involved in optimizing care for neurological disorders, such as Parkinson's disease or Tuberous Sclerosis Complex (TSC) [51,52] These papers also discuss issues concerning the transition from childhood to adulthood care. At the same time, good coordination of multidisciplinary care is very relevant. Small scale studies indicate that the negative impact of uncoordinated care in the field of rare genetic diseases can be high for the patients and their families [53]. In the field of genomic testing in undiagnosed patients, e.g. acute pediatric care setting, continuous reviewing of the multidisciplinary process has proven to be efficient, rapid and cost effective [54].

In the "genomics first" era, the diagnostic interpretation of variants in known genes often requires additional functional tests to confirm pathogenicity. This step can be relatively straight forward for metabolic disorders, with known pathways and available biological/biochemical tests [55], but is less obvious in the heterogeneous field of neurodevelopmental disorders, including MCD.

At the Rotterdam center, the diagnostic tests for MCD are WES-based panels or individual gene sequencing. The diagnostic pipeline applies the internationally accepted 2015 criteria for genomic variant classification [56,57]. The panel is updated twice per year (the latest 2021 release is shown in Supplementary Table 1). In our experience with WES-based MCD diagnostic panels, post-test interpretation of VUS is accomplished within regular multidisciplinary team meetings, including lab specialists, clinical geneticists, neurologists and scientists, where variants are correlated with the patient phenotype and confirmatory functional tests are chosen; this approach has proven to increase the total diagnostic yield (Fig. 2). The total percentage of diagnoses may seem lower than in other cohorts [58], but the test requests came from many different referring clinicians and often clinical data were not available. The diagnoses after evaluation refer to samples of patients referred from the ErasmusMC, with post-genotype revision of clinical and imaging data. We are also faced with the observation that the ACMG criteria of variant classification do not always match phenotype prediction. An example is represented by truncating, i.e. loss of function pathogenic variants, which are deleterious (i.e. pathogenic) according to the ACMG criteria, but do not correlate to disorders caused by gain of function pathogenic variants. A paradigmatic example is PIK3CA-related megalencephaly (M-CAP and PROS, [59]).

Besides the diagnostic application, routine genomic analysis, e.g. WES in trio or extended family, still has the potential to lead to discovery of genes for new Mendelian disorders. In this case, the border between diagnosis and research gets blurred and the next steps require on the one hand complete information and written consent from the undiagnosed patients (family) undergoing genome-wide analysis, and on the other hand a grounded consensus within a multidisciplinary team for adoption of sounded functional confirmatory tests. High flexibility may be required by such a team, which needs to be aware of, and open to collaboration among different disciplines, different centers, different technologies, sometimes limited to remote-type of contacts and exchanges [60,61].

An important role in data sharing and in the identification of new genes for Mendelian disorders is fulfilled by online shared platforms, such as Genematcher [16]. Subscribers of such platform can call for matches based on gene name and inheritance mode. Another model is followed within the European Reference Networks, such as Ithaca (Network on Intellectual Disability and Congenital Malformations), where member organizations can place

Type of test	Tested samples	Direct diagnosis	Additional diagnoses after evaluation	Total diagnoses
Sanger (single gene)	1227	89 (7,2%)	35 (2,8%)	124 (10%)
Targeted NGS (103 genes)	251	18 (7,2%)	12 (4,8%)	30 (12%)
WES CRE (> 200 genes)	108	8 (7,4%)	12 (11,1%)	20 (18,5%)
Total yield	1586	115 (7,2%)	59 (3,7%)	174 (11%)

Fig. 2. Diagnostic yield of genomic tests for MCD between 2012 and 2019 at the ErasmusMC in Rotterdam. The upper panel indicates the total number of the samples processed by the different techniques. The samples came from many different national and international referring clinicians and clinical data were not always available. Targeted NGS refers to the custom made panel run on the Illumina MiSeq platform. WES CRE refers to the exome-based panels. Whole-exome sequencing (WES) was performed on the Agilent Sure Select platform (Clinical Research Exome Capture), run on HiSeq (101bp paired-end, Illumina). The diagnostic certified pipeline is described in Ref. [56].

calls for collaborative clinical research on developmental disorders and reach all participants having a "match" on a novel gene or phenotype (https://ern-ithaca.eu/about-ern-ithaca/ithaca-members/ [73]). Some other platforms, such as GenomeConnect (https://www.clinicalgenome.org/genomeconnect/for-patients-genomeconnect/ [74]) are curated by patient organizations and circumvent the privacy issues involved in online patient registries, but are accessible to researchers and health care professionals as well. Participants of the Neuro-MIG network, dedicated to MCD research and belonging to different disciplines, follow a similar approach in collaborative research (www.Neuro-MIG.org).

The examples reported in this paper show different levels of multidisciplinary interaction that may be required during the process of gene discovery. The discovery of the three MCD genes was in all cases driven by diagnostic questions and was achieved through multidisciplinary collaboration. In all cases, the multidisciplinary setting was already established before and not chosen ad hoc. The regular international expert MRI review session has been organized by professor Dobyns for many years and has already proven to be invaluable. The Neuro-MIG network organizes similar sessions. Dysmorphology review sessions are weekly routine in any clinical genetics center and similar sessions are organized on regular basis during international congresses, or during dedicated courses, i.e. Euro-Dysmo Club (http://www.unicampus.it/eng/current/eighth-european-course-in-clinical-dysmorphology-eurodysmoclub) or the two-yearly Joint Dutch-UK Clinical Genetics meeting, or dysmorphology workshops during the yearly ESHG congress [75]. In her perspective, Dian Donnai concludes that the gremium of clinical geneticists corresponds to a "broad church" bringing together "several distinct tribes"; she breaks a lance in favor of multidisciplinary attendance of European Genetics conferences, and particularly the dysmorphology sessions [62]). Interaction with child neurologists at these sessions seems then more than warranted in the field of MCD.

A long-lasting tradition at the ErasmusMC brings together scientists from the department of Clinical Genetics, Cell Biology and Neuroscience to meet on research topics, allowing regular and informal exchange of knowledge. This interaction has been instrumental to the exploration of RNA-seq in gene discovery and it represents a model applicable in other centers.

In conclusion, all families involved in our studies have been counseled and clinical management has been adapted whenever possible. At the local level, interaction between neurologists, clinical and laboratory geneticists was essential to determine the test of choice and for variant interpretation. But the variant interpretation would not have led anywhere if the search for additional matches would have not been extended to observation of multiple patients with identical physical features (ex. *INTS1*) or of identical brain imaging through international expert remote meetings (ex.

MACF1). The clinical evidence for a phenotype segregation within an extended pedigree, in the absence of a causal variant, would have remained a mere observation of no clinical use, without the application of unexplored techniques like RNA-seq finally pointing to the hidden genomic pathogenic variant (ex. *SMPD4*). These examples indicate that participation of researchers in the multidisciplinary team involved in the care of MCD patients not only supports scientific achievements, but has also the potential to improve the diagnostic yield.

The mission of the European Network on Brain Malformations, Neuro-MIG (COST Action CA16118; www.neuro-mig.org [76]) has been to establish a pan-European collaborative group including scientists and clinicians from different disciplines working together in the field of brain malformations and patient care. Network members are convinced by this experience that the future of MCD research is bound to such interdisciplinary collaboration.

We have explored the application of RNA-seq to gene discovery. In support, recent cohort studies indicate that this technique can solve up to 15—36% of undiagnosed disease cases, most of which concern neurodevelopmental disorders [44,47,48]. The prediction is that, in the diagnostic arsenal of neurodevelopmental disorders, together with other functional tests (e.g. pathway-specific epigenetic signatures), RNA-seq will have a complementary value for the interpretation of rare non coding variants, when whole genome sequencing will replace WES, and protocols will be developed to include it in the routine multiomics diagnostics of Mendelian disorders [63].

Beyond the aims of this paper, dedicated to the scope of gene discovery and neurodevelopmental disorders, we feel that coordinated multidisciplinary interaction should also be the base to continue the follow up of any rare disease (e.g. joint clinics and expertise centers), aiming at understanding natural history, disease mechanisms and development of targeted therapies.

Declaration of competing interest

The authors report no conflict of interest.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpn.2021.09.006.

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