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Variants in nuclear factor I genes influence growth and development

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Abstract

The nuclear factor one (NFI) site-specific DNA-binding proteins represent a family of transcription factors that are important for the development of multiple organ systems, including the brain. During brain development in mice, the expression patterns of *Nfia*, *Nfib*, and *Nfix* overlap, and knockout mice for each of these exhibit overlapping brain defects, including megalencephaly, dysgenesis of the corpus callosum, and enlarged ventricles, which implies a common but not redundant function in brain development. In line with these models, human phenotypes caused by haploinsufficiency of *NFIA*, *NFIB*, and *NFIX* display significant overlap, sharing neurodevelopmental deficits, macrocephaly, brain anomalies, and variable somatic overgrowth. Other anomalies may be present depending on the *NFI* gene involved. The possibility of variants in *NFI* genes should therefore be considered in individuals with intellectual disability and brain overgrowth, with individual NFI-related conditions being differentiated from one another by additional signs and symptoms. The exception is provided by specific *NFIX* variants that act in a dominant negative manner, as these cause a recognizable entity with more severe cognitive impairment and marked bone dysplasia, Marshall–Smith syndrome. *NFIX* duplications are associated with a phenotype opposite to that of haploinsufficiency, characterized by short stature, small head circumference, and delayed bone age. The spectrum of NFI-related disorders will likely be further expanded, as larger cohorts are assessed.

Martin Zenker and Jens Bunt should be considered joint first author.

KEYWORDS
intellectual disability, macrocephaly, NFIA, NFIB, NFIX, nuclear factor one

1 | INTRODUCTION

The nuclear factor one (NFI) family of DNA binding proteins was first identified as a regulator of viral replication (Nagata, Guggenheimer, Enomoto, Lichy, & Hurwitz, 1982), but its members were subsequently identified as important transcription factors (Chen, Lim, Richards, & Bunt, 2017; Harris, Genovesi, Gronostajski, Wainwright, & Piper, 2015; Murtagh, Martin, & Gronostajski, 2003). The proteins, previously also known as CCAAT box-binding transcription factors or TGGCA-binding proteins (Borgmeyer, Nowock, & Sippel, 1984), have four members in vertebrates: NFIA, NFIB, NFIC, and NFIX. They share an N-terminal DNA binding and dimerization domain coded by exons 2 and 3, which is highly conserved between family members as well as between species (Kruse & Sippel, 1994; Rupp et al., 1990; Santoro, Mermod, Andrews, & Tjian, 1988). Consequently, all the four members bind to the same palindromic DNA binding motif as hetero- and homodimers (Gronostajski, Adhya, Nagata, Guggenheimer, & Hurwitz, 1985; Hennighausen et al., 1985; Jolma et al., 2013; Kruse & Sippel, 1994; Leegwater, van Driel, & van der Vliet, 1985). The NFI proteins can also bind to half sites, but with lower affinity (Meisterernst, Gander, Rogge, & Winnacker, 1988). In contrast, the C-terminal transactivation and repression domains differ between the members, potentially providing differential binding to other protein partners. Moreover, each member has multiple isoforms due to alternative splicing (Fletcher, Jenkins, Copeland, Chaudhry, & Gronostajski, 1999). Together, these features of the NFI genes mean that they are promiscuous DNA binding proteins with many different isoforms and identifying specific functions for a given family member or isoform is therefore complex.

2 | MOUSE STUDIES

Expression of the *NFI* genes has been studied in various tissues and cell types (reviewed by Gronostajski (Gronostajski, 2000)), with most of our knowledge being derived from analyses in mice. Based on *in situ* hybridization on mouse embryos, each family member has a unique but overlapping expression pattern during neural development (Chaudhry, Lyons, & Gronostajski, 1997), with *Nfia*, *Nfib*, and *Nfix* expressions being most prominent within the dorsal telencephalon and cerebellum. In the dorsal telencephalon, *Nfia*, *Nfib*, and *Nfix* are first expressed at embryonal day (E)11 within radial glia (Campbell et al., 2008; Mason, Piper, Gronostajski, & Richards, 2009; Plachez et al., 2008). They continue to be expressed in radial glia and ependymal cells, which arise from radial glia subsequent to the generation of neurons and glia (Chen et al., 2017; Vidovic, Davila, Gronostajski, Harvey, & Piper, 2018). The expression patterns of the NFIA and NFIB proteins are initially similar, producing a high caudomedial to low rostro-lateral gradient in the developing dorsal telencephalon in mice (Bunt, Lim, Zhao, Mason, & Richards, 2015). During neurogenesis, intermediate progenitors show reduced expression of NFI proteins, whereas neurons of the deeper cortical layers exhibit higher expression (Bunt et al., 2015; Plachez et al., 2008). This neuronal expression persists into adulthood (Chen, Harris, et al., 2017). Similarly, NFIA and NFIB are expressed in most astrocytes, while NFIA is the primary NFI expressed in oligodendrocytes (Chen, Harris, et al., 2017). In contrast, NFIX expression is mainly restricted to neurons and is greater in the more superficial layer of the cortex, whereas glial expression is more limited (Chen, Harris, et al., 2017). NFIX expression is higher in hippocampal intermediate progenitor cells, although it is also expressed by radial glial cells (Harris et al., 2018).

These three genes are essential for brain development, as knockout mice for any of them display severe brain phenotypes (Supporting Information Table S1) (Bunt et al., 2015; Campbell et al., 2008; Chen, Harris, et al., 2017; das Neves et al., 1999; Driller et al., 2007; Fraser et al., 2016; Lu et al., 2007; Plachez et al., 2012; Plachez et al., 2008; Shu, Butz, Plachez, Gronostajski, & Richards, 2003; Steele-Perkins et al., 2005). No apparent brain phenotype has been observed in the only *Nfic* knockout mouse model currently available (Steele-Perkins et al., 2003), although this gene is expressed at low levels within the developing nervous system (Chaudhry, Vitullo, & Gronostajski, 1998). The brain phenotypes shared between mice lacking *Nfia*, *Nfib*, or *Nfix* include megalencephaly, enlarged ventricles and/or hydrocephalus, malformation of the hippocampus, and dysgenesis of the corpus callosum (Figure 1). In addition to the brain phenotypes, each mouse model also displays other defects unique to that family member (Figure 1a): *Nfia* knockout mice demonstrate urinary tract and kidney anomalies (das Neves et al., 1999; Lu et al., 2007), *Nfib* knockout mice exhibit lung defects (Steele-Perkins et al., 2005), *Nfic* knockout mice have abnormal teeth (Steele-Perkins et al., 2003), and *Nfix* knockout mice have bone abnormalities (Driller et al., 2007). Given these additional organ system defects, *Nfia*, *Nfib*, and *Nfix* knockout mice have very limited perinatal or postnatal viability, which limits long-term studies on these models. The few studies of surviving animals have reported defects such as kidney failure (*Nfia*) and feeding impairment (*Nfic* and *Nfix*), which reduce growth. Studies of heterozygous animals have been limited, although they seem to display an intermediate phenotype (Driller et al., 2007; Harris et al., 2013; Steele-Perkins et al., 2005). Recently, however, conditional deletion mouse models have been generated to overcome the lethality and have allowed study of the role of NFI proteins in specific organs and cells in isolation (Chang et al., 2013; Fraser et al., 2016; Schanze et al., 2018).

Analyses of knockout mice have demonstrated that the shared cortical defects of *Nfi*-deficient mice originate from the delayed differentiation of radial glial cells (Figure 1b,c). Compared with wild-type embryos, each knockout strain exhibits delayed neurogenesis and

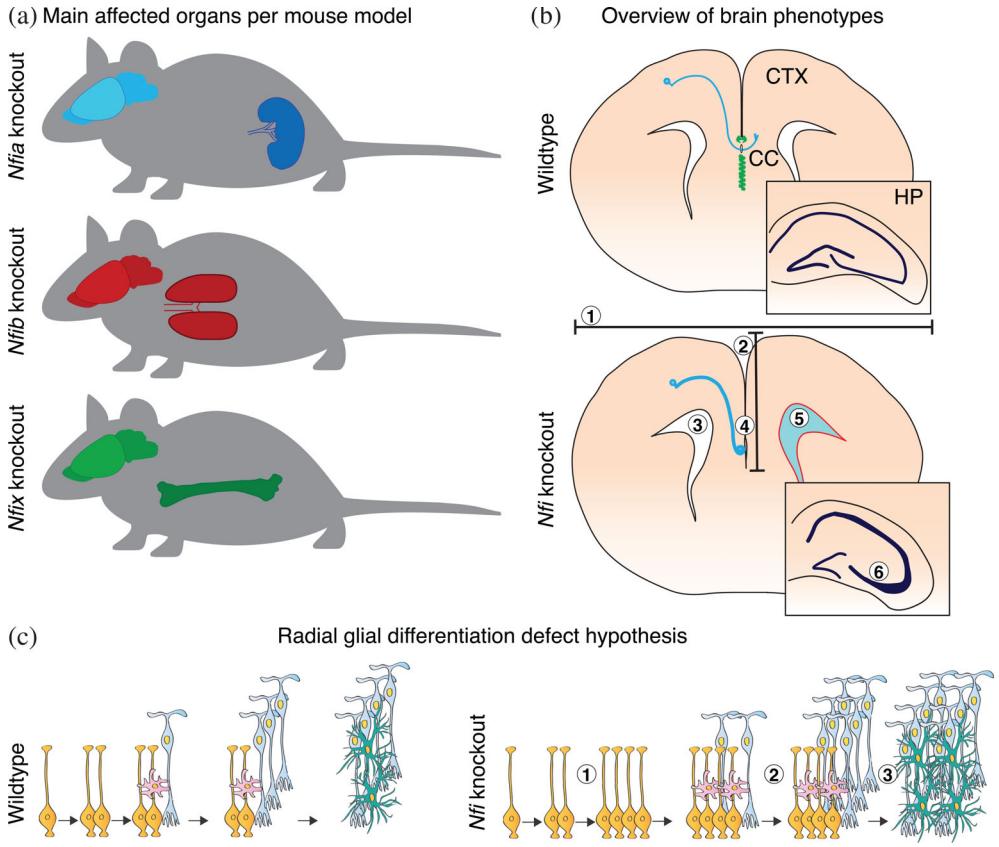

FIGURE 1 Overview of most prevalent phenotypes observed in *Nfi* knockout mice. (a) Although having very limited perinatal or postnatal viability, end stage embryos or postnatal *Nfia*, *Nfib*, and *Nfix* animals all display severe brain malformation, as well as *Nfi* family member-specific defects: renal and urinary tract malformation (*Nfia*), lung defects (*Nfib*), and bone/skeletal muscle abnormalities (*Nfix*). Minor abnormalities, such as eye-opening defects, as well as the *Nfic* knockout phenotype are presented in Supporting Information Table S1. (b) Based on analyses of late embryonic and early postnatal knockout animals as well as heterozygous and conditional models, *Nfia*, *Nfib*, and *Nfix* deletion results in a very similar phenotype in the dorsal telencephalon. Compared with wild-type littermate, the cerebral cortex (CTX) is enlarged (1), resulting in megalencephaly. During development, the cingulate cortex in particular displays lateral expansion (2) and the lateral ventricles are enlarged. (3) In *Nfia* and *Nfib* knockout mice, the corpus callosum (CC) is absent due to the absence of midline remodeling by the midline zipper glia (4), resulting in the callosal axons projecting parallel to the midline and forming Probst bundles. In both *Nfia* and *Nfix* knockout mice, postnatally surviving animals are prone to developing hydrocephalus (5), which for *Nfix* is associated with a differentiation defect of the radial glia into ependymal cells. All three *Nfi* knockout models have a severely malformed hippocampus (HP) with a reduced dentate gyrus (6). (c) Proposed model for the defects in the dorsal telencephalon that occur in *Nfi* knockout mice. Compared with wild-type embryos, the neural progenitors named radial glia (orange) display a delay in differentiation (1). As a result, in early development more progenitors are generated, at the expense of intermediate progenitors (pink) and neurons (blue). Although neurogenesis and gliogenesis are delayed, these processes otherwise proceed normally (2). However, more neurons and glia (green) are generated, resulting in a larger cerebrum (3)

gliogenesis as a consequence of delayed radial glia differentiation (Barry et al., 2008; Bunt et al., 2017; Harris et al., 2016; Piper et al., 2009; Piper et al., 2014). Hence, the radial glia remain self-renewing for an extended period of time, before switching to asymmetric cell divisions to generate differentiated progeny. This extended self-renewal results in an increase in the total number of neural progenitors in the developing dorsal telencephalon (Barry et al., 2008; Betancourt, Katzman, & Chen, 2014; Heng et al., 2014). This could contribute to the structural enlargement of the lateral ventricles to accommodate the additional progenitors, as well as brain overgrowth (Bunt et al., 2015; das Neves et al., 1999; Lu et al., 2007; Piper et al., 2014). Although *Nfia* and *Nfib* knockout mice die at birth, *Nfix* knockout mice can survive until postnatal day 20 on a C57Bl/6 background. These knockout mice present with enlargement of the cingulate cortex and elevated numbers of neurons and glia within the dorsal telencephalon. In line with a delay in differentiation, cortical radial glia eventually differentiate in the absence of *Nfix* and produce more postmitotic progeny. *Nfix* knockout mice also exhibit dysmorphic hippocampal development, and commonly develop hydrocephalus around postnatal day 10, likely due to abnormal differentiation of radial glia into ependymal cells (Driller et al., 2007; Shu et al., 2003; Vidovic et al., 2015; Vidovic et al., 2018). Due to the lethality, feeding difficulties, and frequent hydrocephalus, overgrowth is difficult to assess in any of the *Nfi* homozygous knockout strains. However, cerebral overgrowth is observed in mice with heterozygous knockout of *Nfix* as well as in those with cortical-specific deletion of *Nfib* (Oishi et al., 2019; Schanze et al., 2018).

Independent of the cerebral defects, complete or partial agenesis of the corpus callosum occurs in both *Nfia* and *Nfib* knockout mice (das Neves et al., 1999; Gobius et al., 2016; Steele-Perkins et al., 2005). This defect originates from a delay in interhemispheric remodeling due to defects in midline glia development (Gobius et al., 2016). As a result, callosal axons are unable to cross the midline and instead form Probst bundles (Shu et al., 2003; Steele-Perkins et al., 2005). Hence, with the presence of *Nfi* in these specific radial glia, the midline and the corpus callosum form normally (Schanze et al., 2018). In line with their function in the forebrain, the three *Nfi* genes are also important in cerebellar and spinal cord development, with knockout animals displaying delayed differentiation of progenitor cells in these regions (Deneen et al., 2006; Fraser et al., 2019; Kang et al., 2012; Kilpatrick, Wang, Gronostajski, & Litwack, 2012; Kumbasar, Plachez, Gronostajski, Richards, & Litwack, 2009; Matuzelski et al., 2017; Wang et al., 2004; Wang, Crandall, Litwack, Gronostajski, & Kilpatrick, 2010).

Currently, little is known about the functional consequences of abnormal brain development evident in *Nfi*-deficient mice. At this point, behavioral studies have only been performed on adult *Nfix* heterozygous and conditional knockout animals (Harris et al., 2013; Harris et al., 2018; Oishi et al., 2019; Zalucki et al., 2018). In *Nfix* heterozygous mice, the overall brain size is increased by approximately 15%, with all structures being enlarged, including the lateral ventricles (Oishi et al., 2019). In scale with the neocortex, the corpus callosum is also larger and all forebrain commissures display altered properties and connectivity based on diffusion tensor MRI analyses (Oishi et al., 2019). These animals breed normally and display normal motor function, suggesting limited cerebellar defects despite the altered size. Although their anxiety-related behavior is also normal, their spatial learning and memory are impaired. This can be at least partly attributed to impaired neurogenesis in the dysmorphic hippocampus, as similar behavioral changes are observed in conditional mice with postnatal deletion of *Nfix* from hippocampal neural stem cells (Harris et al., 2018; Zalucki et al., 2018).

Outside the nervous system, the role of NFIs in timing and regulating cellular differentiation appears conserved. For instance, NFIB is essential for the differentiation and maturation of lung epithelium, as evidenced by the immature lungs and respiratory defects reported in *Nfib* knockout mice (Hsu et al., 2011; Steele-Perkins et al., 2005). Although the role of NFI proteins has also been investigated in other cell and tissue types on a molecular or cellular level, including hematopoiesis and hair follicles, it remains to be determined whether and how they translate to clear phenotypes within the mouse models.

Although studied since the 1980s, the NFI regulatory pathways still require further elucidation. In particular, our knowledge concerning the upstream regulation of the *NFI* genes is limited. Although transcription factors including SOX9, BRN2, NOTCH, NFkB, and LHX2 have been implicated (Deneen et al., 2006; Fane et al., 2017; Glasgow et al., 2017; Kang et al., 2012), understanding the precise regulation is complicated by post-transcriptional regulation by various micro-RNAs and RNA binding proteins such as DROSHA (Glasgow et al., 2013; Rolando et al., 2016; Tsuyama et al., 2015). As expected based on their shared DNA binding motifs and similar brain phenotypes, the NFI-regulated genes in knockout models overlap in the developing cortex (Bunt et al., 2017; Harris et al., 2016). The NFI proteins function in combination, as the number of deleted alleles seems to determine the severity of the phenotype (Bunt et al., 2017; Harris et al., 2016). It remains to be determined whether this holds true within later stages of development or within other tissues. Although some *in vitro* studies have reported similar abilities of NFI proteins to activate promoters (Bachurski, Yang, Currier, Gronostajski, & Hong, 2003; Brun et al., 2009; Gobius et al., 2016), others have reported differences between family members and splicing isoforms (Mukhopadhyay, Wyszomierski, Gronostajski, & Rosen, 2001; Perez-Casellas et al., 2009; Singh et al., 2011).

Traditionally, NFI research has focused on NFI binding to target sites in the promoters of genes. More recent studies in development and cancer have revealed that NFI proteins can also act as epigenetic regulators and that binding is associated with open, active chromatin and active enhancers (Denny et al., 2016; Fane, Harris, Smith, & Piper, 2017; Hiraike et al., 2017; Martynoga et al., 2013; Shin et al., 2016; Willi, Yoo, Wang, Trajanoski, & Hennighausen, 2016). This more global regulation of gene expression might be important for the multiple functions that NFI proteins play in the development of various tissues.

3 | HUMAN DISORDERS CAUSED BY NFI VARIANTS

3.1 | NFIA

The human *NFIA* gene (MIM 600727) has its cytogenetic location on 1p31.3. Lu and coworkers were the first to propose that *NFIA* haploinsufficiency was responsible for a developmental syndrome including brain anomalies (malformed corpus callosum, ventriculomegaly, and other abnormalities) and urinary tract defects based on five individuals with translocations or interstitial deletions disrupting the *NFIA* locus (Lu et al., 2007). However, in all these individuals, a contribution of position effects or other genes affected at translocation breakpoints or by microdeletions could not be excluded. Subsequently, a 254 kb intragenic deletion encompassing exons 4–11 of *NFIA* was reported in a young adult with intellectual disability, bipolar disorder, macrocephaly, small corpus callosum, hydrocephalus, and scoliosis (Mikhail et al., 2011), while the first report of a *de novo NFIA* point mutation appeared in 2012 as part of a series of children with autism spectrum disorder (Iossifov et al., 2012). To date, 15 individuals from 9 unrelated families with genetic variants affecting only *NFIA* have been reported with sufficient clinical data, to which we add two unreported individuals (Figure 2; Supporting Information Table S2) (Revah-Politi et al., 2017). In six individuals, the variant was found to have occurred *de novo*, four were familial with transmission of the variant from an affected parent, and in one the segregation remained unclear (Mikhail et al., 2011). All *NFIA* changes have been detected by either microarray (deletions) or exome sequencing (point mutations). The observed genetic changes included three nonsense variants, two

FIGURE 2 Clinical facial phenotypes of individuals with variants in *NFI* family members. (a) *NFIA* haploinsufficiency in a 17-year-old boy. Note the long face, prominent forehead (OFC 90th centile), mildly underdeveloped midface, thin upper vermillion, and hearing aids. (b) *NFIB* haploinsufficiency in a 4-year-old boy. Note the frontal upsweep, broad and prominent forehead (OFC > 97th centile), mild asymmetry, enlarged nares, long and smooth philtrum, and thin upper vermillion (left panel published in Schanze et al., 2018). (c) *NFIX* haploinsufficiency (Malan syndrome) in a 6-year old boy. Note the long face, prominent forehead (OFC >99th centile), underdeveloped lateral part of the eyebrows, deeply set eyes, short nose, upturned nasal tip, pointed chin, and prominent ears (left panel published in (Priolo et al., 2018); courtesy of Dr Jan Liebelt, Adelaide, South Australia, Australia). (d) Marshall–Smith syndrome (presumed dominant-negative acting *NFIX* variant) in a 4-year-old boy. Note the prominent forehead (OFC 25th centile), proptosis, wide mouth, and everted vermillion of the lower lip. (e). *NFIX* duplication in a 1-year 10-month-old boy. Note microcephalic aspect (OFC < 3rd centile), full cheeks, everted vermillion of the lower lip, and large appearing ears (the same individual as patient 9 in Trimouille et al., 2018; courtesy of Dr Aurélien Trimouille, Bordeaux, France). Signed consent for use of photographs for publication was obtained for each individual. OFC: occipitofrontal head circumference

frameshift variants, one missense change, five small deletions affecting solely *NFIA*, and one translocation disrupting *NFIA* (Figure 3, Supporting Information Figure S1). The nature and distribution of point mutations and deletions described thus far strongly suggest haploinsufficiency of *NFIA* as the common pathogenic mechanism in all of these genetic variants.

Several individuals have been reported with microdeletions involving *NFIA* and a variable number of flanking genes (Chen et al., 2011; Ji, Salamon, & Quintero-Rivera, 2014; Koehler et al., 2010; Labonne et al., 2016; Schirwani, Smith, & Balasubramanian, 2018). In a few others, translocations in which additional genes on the translocated chromosome were disrupted or possibly subject to position effects on either sides of the breakpoints of both chromosomes were reported that may have contributed to the phenotype (Lu et al., 2007; Shanske, Edelmann, Kardon, Gosset, & Levy, 2004). In other individuals, a single additional gene, such as *PTEN* or *RBFOX1* (Revah-Politi et al., 2017; Zhao, 2013), was found to harbor a variant, which may have influenced the phenotype. Furthermore, four reported and two unpublished individuals are known to us for whom insufficient clinical data were available (T. Attie-Bitach, G. Battista Ferrero and K. Devriendt, personal communications 2019) (Hollenbeck et al., 2017; Krumm et al., 2015). Hence, we did not include the existing data on these individuals in determining the phenotype overview (Table 1). One three-generation family is known to us (J.V.) with a 3.07 kb intronic microdeletion (61,789,065-61,792,138) of *NFIA*. In this family, the child has a phenotype fitting others with an *NFIA* variant, whereas the parent and grandparent do not. As it remains uncertain whether this variant alters gene splicing or expression, we have not included this family in the clinical overview presented here.

The phenotype of isolated *NFIA* haploinsufficiency in the 17 individuals with point mutations and deletions affecting *NFIA* only (core *NFIA* phenotype) is summarized in Table 1. Developmental delay and

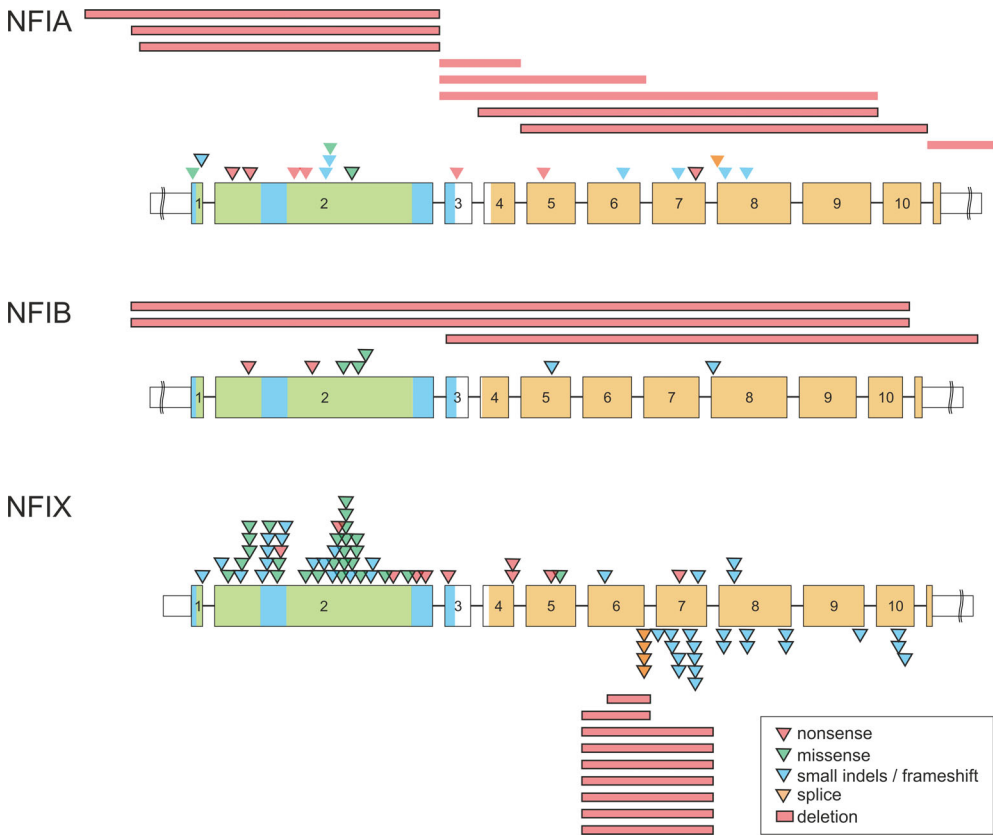

FIGURE 3 Variants in each of the three *NFI* genes, *NFIA*, *NFIB*, and *NFIX*. Exons are to scale, whereas the UTR, introns, and start and end positions of intragenic deletions in introns are not to scale. Mutation types are indicated by specific color-coded symbols. Color coding of exons refers to known functional domains of the respective proteins. Blue background color shows the putative DNA binding and dimerization domains with green color representing the MH1 (MAD homology 1) and the N-terminal DNA binding (DNAbd) domains. The orange background highlights the CAAT-box transcription factor—nuclear factor I (CTF-NFI) domains. For *NFIA*, symbols lacking a black frame represent additional variants reported in the literature (Hollenbeck et al., 2017; Krumm et al., 2015), and others listed as "likely pathogenic" or "pathogenic" variants in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/). For these *NFIA* variants, no information about the associated phenotype is available to verify differences or similarities to the *NFIA*-associated phenotype described here. For *NFIB* and *NFIX*, the displayed variants refer to recently published original and review papers, respectively (Schanze et al., 2018; Priolo et al., 2018) Malan syndrome-associated variants are shown above and Marshall–Smith syndrome-associated variants are shown below the schematic for the *NFIX* gene. Isoform references: NFIA, ENST00000403491.7; NFIB, ENST00000380953.5; NFIX, ENST00000592199.5

intellectual disability (mild to moderate) and brain anomalies represent the most common clinical features. Affected individuals display a variable and wide spectrum of abnormal brain functions, including autism spectrum disorder, behavioral abnormalities, psychiatric symptoms, and seizures. Among the abnormal findings observed with brain imaging, small or absent corpus callosum as well as ventriculomegaly are present in a majority of individuals. Chiari malformation, likely as a reflection of cerebral overgrowth, has been observed in several instances. Pre- and postnatal macrocephaly is very common, whereas macrosomia and tall stature have only been observed only in a minority of individuals. Urinary tract anomalies, which are also found in *Nfia*-deficient mice, were initially suggested to be a key symptom of *NFIA* haploinsufficiency (Revah-Politi et al., 2017), but significant urinary tract abnormalities were present less than 30% of cases included in our review. Craniosynostosis has been reported in four cases, but three individuals affected by craniosynostosis were members of one family (Nyboe, Kreiborg, Kirchhoff, & Hove, 2015), raising the possibility of additional genetic modifiers in this family. Although no recognizable craniofacial gestalt has been defined, many affected individuals do have minor morphologic signs (Figure 2). A prominent forehead is the most commonly observed anomaly (corresponding to the frequent finding of macrocephaly). While neurodevelopmental defects, macrocephaly with ventriculomegaly, and callosal hypoplasia or dysgenesis have clearly emerged as the key features of *NFIA* haploinsufficiency, the limited number of clinically well-described cases still leaves considerable uncertainty regarding the full clinical spectrum and reporting of additional cases is strongly supported.

At least 20 variably sized microdeletions (≤5 Mb), including in the *NFIA* locus as well as additional genes (1p32p31 deletions; MIM 613735), have recently been described and reviewed (Prontera et al., 2017; Revah-Politi et al., 2017). Next to variable neurodevelopmental deficits, the majority of affected individuals demonstrate macrocephaly, corpus callosum anomalies, and wide ventricles, thereby providing evidence that *NFIA* is the critical gene for the brain

TABLE 1 Major clinical characteristics of the phenotypes caused by variants in the individual *NFI* genes

		NFIA	NFIB	NFIX		
				Malan syndrome[a]	Marshall–Smiths syndrome[b]	Dup[c]
Total included individuals		17	13	80	57	10
Prenatal:	length increased	0/16	1/12	7/45	2/26	0/6
	birth weight (mean)[d]	(3860 g)[f]	3360 g	3460 g	3125 g	3140 g
Postnatal:	length/height increased[g]	5/11	2/11	44/79	2/36	0/10
Adult height (mean)[e]	males	(167.5 cm)[f]	(182.8 cm)[f]	(184.7 cm)[f]	(140 cm)[f]	NA
	females	(154.5 cm)[f]	NA	(158.0 cm)[f]	(127 cm)[f,h]	NA
Prenatal:	macrocephaly	6/6	2/5	19/46	1/56	0/4
	birth OFC (mean)[d]	(39.6 cm)[f]	37.1 cm	36.2 cm	35.7 cm	(33.5 cm)[f]
Postnatal:	macrocephaly	13/15	12/12	60/79	1/40	0/2
	adult OFC (mean)[e]	(59.1 cm)[f]	(61.5 cm)[f]	60.6 cm	NA	NA
Body build:	slender	1/11	3/10	46/78	0/57	0/10
	obese	3/11	1/10	3/78	0/36	NA
Developmental delay[i]	+/++/+++	15/17+ or ++	13/13 + or ++	80/80 + or ++	39/39 ++ or +++	9/9 + or +++
Autism		2/11	3/9	23/74	NA	NA
Seizures		3/11	0/11	21/79	4/38	NA
Hypotonia		5/7	7/11	57/75	12/28	NA
Small/absent corpus callosum		15/16	3/8	14/63	8/39	NA
Wide ventricles/hydrocephaly		12/16	1/7	17/63	2/39	NA
Frontal lobe anomaly		3[j]	1[j]	2[j]	NA	NA
Long face		1/10	NA	67/79	3/57	0/4
Facial asymmetry		4/10	6/9	5/42	4/36	0/4
Craniosynostosis		4/17	NM	0/42	4/57	NM
Prominent forehead		6/10	6/9	77/79	53/54	0/4
Thin eyebrows		2/6	1/9	15/66	5/35	0/4
Proptosis		0/10	0/9	1/78	55/56	0/4
Underdeveloped midface		2/10	4/9	1/79	38/42	0/4
Anteverted nares		1/10	2/9	43/76	44/53	0/4
Thin vermillion upper lip		6/10	3/9	42/63	1/35	0/4
Low-set ears		5/10	0/9	8/42	13/40	NA
Proximally placed thumbs		4/7	0/9	1/42	0/27	NA
Abnormal bone maturation		NA	NA	40/50	57/57	5/66 (delay)
Significant urinary tract anomalies[k]		3/15	0/10	1/42	2/36	NA

[a]All variants lead to haploinsufficiency.
[b]All variants lead to altered protein formation.
[c]Reported duplications vary in size from to 3.1 Mb to 479 kb, with a 422 kb minimal region of overlap which contains 16 genes.
[d]Only at term born newborns (38–42 weeks) used.
[e]Only individuals 16 year and older used.
[f]Only a small number of data available.
[g]Length/height ≥ 2SD for age.
[h]Reliability limited due to scoliosis.
[i]+ mild cognitive impairment (IQ = 50–70); ++ moderate cognitive impairment (IQ = 35–50); +++ severe cognitive impairment (IQ < 35).
[j]Only positively scored findings mentioned as not all MRI scan were available for personal evaluation.
[k]Excluding one or two small cysts.
Abbreviations: OFC, occipitofrontal head circumference; NA, no data available; NM, not mentioned.

phenotype of 1p32p31 deletions. Chiari malformation, urinary tract abnormalities, and craniofacial anomalies occur in microdeletions at similar frequencies to intragenic variants (Revah-Politi et al., 2017). However, genes flanking *NFIA* are also likely to play a role in the 1p32p31 deletion syndrome phenotype, as individuals with larger deletions tend to have more severe neurodevelopmental phenotypes (Revah-Politi et al., 2017). No specific additional signs or symptoms are known to occur regularly in individuals with deletions involving other genes. With larger deletions (>5 Mb), other anomalies have been reported such as craniosynostosis, moyamoya angiopathy (Prontera et al., 2017) and ambiguous genitalia (Chen et al., 2011), but it generally remains difficult to relate individual features to one gene in such deletions, and the possible contributions of flanking genes to the 1p32p31 microdeletion syndrome currently remain speculative.

3.2 | NFIB

The human *NFIB* gene (MIM 600728) has its cytogenetic location on 9p23p22.3. *NFIB* has only recently been related to human developmental disorders, when 13 individuals from 10 unrelated families with variants affecting solely *NFIB* were reported (Schanze et al., 2018). Disruption of *NFIB* by a balanced translocation was reported in one individual (Aristidou et al., 2018), but the clinical data were limited to define a clear phenotype. The observed genetic changes included three nonsense variants, three missense variants, one frameshift variant, and three small deletions affecting only *NFIB* (Figure 3). All genetic abnormalities have been detected by either microarray (deletions) or exome sequencing (point mutations). Haploinsufficiency as the underlying mechanism was evidenced by the nature and distribution of point mutations and deletions. The missense changes that affected the highly conserved DNA-binding domain of the protein were demonstrated to confer loss of transcriptional activity in an *in vitro* reporter assay (Schanze et al., 2018). Moreover, for two of these variants, pathogenic missense changes in the corresponding codons of *NFIX* have been observed in individuals with Malan syndrome (Supporting Information Figure S1), which is the phenotype associated with haploinsufficiency of *NFIX* (*vide infra*). Pathogenic *NFIB* variants occurred *de novo* in seven affected individuals, and were inherited from a similarly affected parent in two. In three families the segregation remained unclear.

The phenotype of isolated *NFIB* haploinsufficiency in individuals with point mutations and deletions affecting only *NFIB* is summarized in Table 1. The data suggest a core *NFIB* phenotype consisting of developmental delay and mild to moderate intellectual disability, macrocephaly and non-specific craniofacial anomalies (Figure 2). Many affected individuals exhibit behavioral abnormalities and psychiatric disorders. Structural brain anomalies are less consistent but resemble those seen in *NFIA* haploinsufficiency, especially in the underdevelopment of the corpus callosum. Postnatal macrocephaly is a very common finding, but prenatal skull growth can also be normal with other growth parameters being elevated in only a small minority of individuals. Pulmonary defects have not yet been observed in individuals with *NFIB* haploinsufficiency. As for *NFIA*, the number of reported individuals with *NFIB* variants is still too small to delineate the clinical spectrum completely.

Several 9p23p22.2 microdeletions encompassing *NFIB* and neighboring genes have been reported (Sajan et al., 2013; Schanze et al., 2018). Despite the varying size (1.5–4.9 Mb) and difference in the position of the deletions, the degree of cognitive impairment of individuals did not significantly differ from that observed in intragenic variants (Schanze et al., 2018; Vissers et al., 2011). A small or absent corpus callosum is a recurrent but inconsistent finding in 9p23p22.2 microdeletions (Sajan et al., 2013; Schanze et al., 2018). It is remarkable that three individuals with larger deletions displayed a similar facial gestalt but had no significant macrocephaly (Schanze et al., 2018). 9p22.3 deletions have previously been reported in individuals with metopic craniosynostosis and neurodevelopmental deficits, and it has been suggested that the deletion of *FREM1* (adjacent to *NFIB*) was the major cause of this phenotype (Vissers et al., 2011). However, it seems unlikely that a recessively acting gene that causes no phenotype in carriers of loss-of-function variants is sufficient to explain the phenotype. This suggests a contribution by other dosage-sensitive genes to the phenotype in individuals with larger deletions, mitigating the macrocephaly. Hence, *NFIB* appears to be a critical gene for the phenotype of 9p23p22.2 microdeletions, but deletion of additional genes likely influences the phenotype. Further work is therefore needed to uncover the mechanism involved.

3.3 | NFIC

To date, no individuals have been reported with an intragenic *NFIC* variant or with a microdeletion or microduplication of chromosome 19p13.3 involving only *NFIC* (MIM 600729). A series of 27 individuals with microdeletions or microduplications of this region have been reported, and in 19 of these *NFIC* was involved (Nevado et al., 2015). Individuals typically demonstrate intellectual disability, macrocephaly, and short stature. Other features include variable facial characteristics, gastroesophageal reflux, syndactylies, and congenital heart malformations. However, this phenotype was found not only in those in whom *NFIC* was included in the deleted or duplicated region but also in those in whom *NFIC* was not included. Dental signs, which form part of the phenotype in the *Nfic* knockout mouse model, have not been reported (Lee et al., 2009; Steele-Perkins et al., 2003). It has been suggested that *PIAS4* in particular is responsible for the phenotype but individuals with imbalances in which *PIAS4* was not deleted showed a similar phenotype (Nevado et al., 2015). It therefore seems likely that both the deleted *PIAS4* and *NFIC* are involved in the phenotype (personal communication, Pablo Lapunzina, December 2018). Based on our current knowledge, the role of *NFIC* dosage sensitivity in humans with 19p13.3 microdeletions and microduplications remains uncertain.

3.4 | NFIX: Malan Syndrome

NFIX (MIM 164005) is located at chromosome 19p13.13. Microdeletions of *NFIX* lead to Malan syndrome, and variants in *NFIX* may

lead to two different disorders, Malan syndrome and Marshall–Smith syndrome (*vide infra*). Malan syndrome (MIM 614753) was initially described in 2010 when *NFIX* variants were first reported in a series of individuals with Marshall–Smith syndrome; three individuals with intellectual disability and overgrowth were also found to have an *NFIX* variant (Malan et al., 2010). Numerous subsequent reports confirmed that this constitutes a separate entity, with a recent review describing 45 newly recognized affected individuals and 35 who had been reported in literature, with either point mutations or 19p13.13 deletions, including the *NFIX* locus (Priolo et al., 2018). The majority of *NFIX* variants were ascertained either by microarray (deletions) or by WES (point mutations), but in a few instances, mutations were also detected by targeted sequencing of *NFIX* in individuals who resembled Sotos syndrome and previously tested negative for *NSD1* mutations (Priolo et al., 2018). Malan syndrome-associated *NFIX* point mutations detected in 56 affected individuals comprised 51 different variants. More than half of these were nonsense and frameshift variants predicting premature stop codons mostly in the 5′ region of the mRNA, presumably leading to nonsense-mediated mRNA decay. Twenty-three different missense variants were reported, most of which affect highly conserved residues in the DNA binding and dimerization domain (Figure 3, Supporting Information Figure S1). Intragenic deletions of one or more exons have not yet been reported in Malan syndrome, but specific exon deletions are associated with Marshall–Smith syndrome (*vide infra*). Malan syndrome typically occurs sporadically and is caused by a *de novo* mutational event. Exceptionally, familial occurrence has been observed, including two instances of transmission by a mildly affected parent with a proven mosaic status in one family (Priolo et al., 2018; Yoneda et al., 2012), as well as one pair of affected siblings due to presumed parental germline mosaicism (Nimmakayalu et al., 2013).

The main clinical characteristics are the overgrowth, most marked in prenatal and postnatal skull growth, but also frequently evident in increased height and weight (Table 1). The face can be characteristically: long, triangular, with a prominent forehead, everted lower lip, and a prominent chin (Figure 2), but variation is marked. A slender habitus and long hands occur in over 50% of cases, with infrequent pectus formation. An advanced bone age is very common. Recently, it was suggested that a widened aorta occurs with an increased frequency (Priolo et al., 2018), but abnormalities of the internal organs are otherwise infrequent. Neuro-imaging yields normal results in most individuals. Infrequent findings are cortical dysplasia and periventricular heterotopia. Intellectual disability, sometimes mild, but typically moderate to severe, has been observed in all described individuals. Behavioral problems are frequent and are dominated by anxieties, and less frequently aggression toward themselves or others. However, detailed studies are lacking.

A comparison between 24 individuals with microdeletions that included the complete gene and 56 individuals with intragenic *NFIX* variants and a Malan phenotype failed to show differences in growth pattern, cognitive impairment, and facial or skeletal characteristics (Priolo et al., 2018). This indicates that *NFIX* is the critical gene for the phenotype of the 19p13.2 microdeletion syndrome. The exception occurs when individuals exhibit seizures and EEG abnormalities; individuals with intragenic variants or deletions, which contain only *NFIX* and no other genes, have only occassional seizures, whereas 10 out of 14 individuals with a microdeletion that included *CACNA1A* developed seizures (Priolo et al., 2018). This indicates that a microdeletion involving both *NFIX* and *CACNA1A* should be considered a contiguous gene syndrome.

3.5 | NFIX: Marshall–Smith Syndrome

Marshall–Smith syndrome (MIM 602535) was first described in 1971 by Marshall, Smith, and colleagues in two infants with an unusual face, delayed growth and development, and abnormal osseous maturation (Marshall, Graham, Scott, & Smith, 1971). Subsequently, a study of 58 affected individuals demonstrated that the most common manifestations are a marked developmental delay, typical face (high forehead, proptosis, underdeveloped midface, anteverted nares, and retrognathia), respiratory problems especially in infancy and early childhood, hypertrichosis, and the disturbed bone formation which leads to a seemingly advanced bone age in childhood (this led to tagging the entity as an overgrowth disorder despite the lack of overgrowth), decreased growth in height, progressive kyphosis and scoliosis from early puberty, and osteoporosis which continues to progress in adulthood (Figure 2; Table 1) (Shaw et al., 2010). The spinal anomalies can lead to cervical spine compression and spastic tetraparesis. Brain neuro-imaging may show underdevelopment of the corpus callosum, and sometimes wide ventricles, cortical malformations variously described as pachygyria or polymicrogyria, and septo-optic dysplasia (Shaw et al., 2010). Seizures are uncommon. The behavior of the affected individuals is characterized by a marked delay in cognition, motor development, and adaptive functioning, and a friendly and happy demeanor and enjoyment of social interactions (van Balkom et al., 2011).

In 2010, variants in *NFIX* were reported (Malan et al., 2010), which were subsequently found to be limited to exon deletions, indels, and splice site variants that lead to frameshift downstream of exon 5 (Figure 3) (Priolo et al., 2018; Schanze et al., 2014). All variants occurred *de novo*. Functional studies have shown that the mutant NFIX proteins in individuals with Marshall–Smith syndrome are not cleared by nonsense-mediated mRNA decay, have a preserved DNA binding and dimerization domain, and therefore likely act in a dominant negative manner (Schanze et al., 2014).

3.6 | NFIX: Duplications

A small number of individuals have been reported who were found to have a duplication of part of chromosome 19 that includes *NFIX* (Dolan et al., 2010; Trimouille et al., 2018). The duplications vary in size from to 3.1 Mb to 479 kb, with a 422 kb minimal region of overlap which contains 16 genes. The phenotype is remarkable in that it shares intellectual disability with Malan and Marshall–Smith, but the other signs could be summarized as the opposite of Malan syndrome: short stature, small head circumference, and delayed bone age. The

facial characteristics are variable, but highly arched eyebrows, full cheeks, and a thick, everted lower lip have been reported (Figure 2). Several affected individuals had brachydactyly and gastrointestinal motility disorders, one had an underdeveloped aorta, another had an atrial septal defect, and still another had lagophthalmos (Table 1) (Trimouille et al., 2018). No results of neuroimaging have been described. One cannot be sure that the phenotype is mainly caused by duplication of *NFIX* and not of one of the other genes involved, but since the growth pattern and bone development are opposite to those present in individuals with *NFIX* deletions, these *NFIX* duplications may be an example of a mirror phenotype (Lindstrand et al., 2014).

4 | DISCUSSION

The above overview indicates that variations in copy number or intragenic variants of *NFI* genes are emerging as a new family of human (neuro)developmental disorders. The findings document the importance of these genes in human development, particularly in the brain, and their tight physiological regulation.

The phenotypes associated with haploinsufficiency of *NFIA*, *NFIB*, or *NFIX* display a significant overlap, particularly regarding the spectrum of structural and functional brain defects: increased cerebral volume, corpus callosum anomalies, and neurodevelopmental and behavioral impairment. Macrocephaly is consistently seen at high prevalence throughout the *NFI* haploinsufficiency disorders, thereby making the entire group an important differential diagnosis in an individual presenting with macrocephaly and intellectual disability. Clinicians are inclined to consider Sotos syndrome (MIM #117550) in every individual with macrocephaly and intellectual disability, whereas obviously a series of entities can cause this (see this Issue). The NFIs should be included when considering causes of this phenotype. If studied more carefully, typically also the clinical differences are usually obvious (Priolo et al., 2018).

The similarities in the phenotype of the *NFI* haploinsufficiency disorders document the overlapping but non-redundant functions of NFIs during brain development, as suggested by the findings in mouse models. In all three *Nfi* knockout models, radial glia of the forebrain display a similar delay in the switch from the generation of more progenitors to neurogenesis and gliogenesis. As the NFI proteins regulate comparable biological processes and the number of mutated *Nfi* alleles is directly indicative of the severity of the radial glial phenotype (Bunt et al., 2017; Harris et al., 2018), the overall combined levels of NFI proteins might impact the cerebral phenotype. The importance of NFI levels in human brain growth and development is further supported by the mirror phenotype between *NFIX* deletions and *NFIX* duplications. Due to the additive nature of NFI function in the brain, further study is required to determine whether the variability in phenotypes observed between individuals with haploinsufficiency for one of the *NFI* genes is in part also determined by additional variants affecting function or regulation of the other *NFI* family members.

Physical overgrowth is also a characteristic of *NFI* haploinsufficiency but seems less frequent and less significant compared with other entities showing overgrowth with intellectual disability discussed in this Issue. Nonetheless, the NFI proteins show many similarities and interactions with other overgrowth genes. NFI proteins also function as epigenetic modifiers, and physically interact with modifying proteins, including EED, P300, and CREBBP, and NFIB has been reported to regulate the expression of *Ezh2* (Cao et al., 2014; Fane, Chhabra, et al., 2017; Leahy, Crawford, Grossman, Gronostajski, & Hanson, 1999; Piper et al., 2014). Furthermore, within the context of brain development, NFI proteins have been shown to be essential downstream effectors of FGF-FGFR-mediated signaling (Gobius et al., 2016). Due to the lack of appropriate mouse models to investigate postnatal growth and limited information about the NFI expression or function within this biological process, it remains unclear whether the function of the NFI proteins is redundant. In the case of *NFIX* haploinsufficiency (Malan syndrome), the association with tall stature and physical overgrowth appears to be stronger than for the other *NFI* genes. This may reflect a distinct biological function of NFIX, or *NFIX* might be the predominantly expressed family member in this context.

The same could also hold true for other observed differences between the *NFI* haploinsufficiencies. The higher prevalence of a small or absent corpus callosum in individuals with *NFIA* haploinsufficiency compared with Malan syndrome might be related to differences in the abundance or spatial or temporal expression patterns during brain development, particularly as NFIX is less expressed in astrocytes (Chen, Harris, et al., 2017). The intellectual deficits appear to be more severe in *NFIX* haploinsufficiency than *NFIA* and *NFIB* haploinsufficiency, which is also supported by the observation of parent-child transmissions for the latter genes, but not for *NFIX* (the few reported familial cases have been explained by mosaicism in the transmitting parent) (Priolo et al., 2018). This could be indicative of a specific role for NFIX in neurons, as it is the predominantly expressed NFI in these cells, and heterozygous knockout mice have altered brain wiring (Chen, Harris, et al., 2017; Oishi et al., 2019). Regarding the associated physical abnormalities unrelated to brain development, the association of *NFIA* haploinsufficiency and renal and urinary tract anomalies is notable. Although the first description of *NFIA* haploinsufficiency based on five subjects with large genomic aberrations identified significant defects in all three subjects with renal and urinary examination data available (Lu et al., 2007), similar anomalies were only reported for three out of 15 individuals with isolated *NFIA* haploinsufficiency that are reviewed here (Table 1). This association as well as the taller stature observed in Malan syndrome reflect the associations with urinary tract abnormalities and more severe skeletal involvement in *Nfia* and *Nfix* knockout mouse models, respectively (Driller et al., 2007; Lu et al., 2007; Messina et al., 2010). Lung defects as an anomaly specific to *Nfib* knockout mice have not been observed in humans with *NFIB* haploinsufficiency. This could be consistent with the hypothesis that the impact of altered regulation caused by the loss of just one allele of *NFIB* (and other *NFIs*) is greater in the development of the brain than other organs. However, in the *Nfib* mouse model, abnormalities in lung development, although asymptomatic in heterozygotes, are nevertheless detectable by microscopy. Similar

subclinical anomalies could also exist in humans with *NFIB* haploinsufficiency and might be associated with health issues, possibly of later onset and of which we are currently unaware. Although differences between the phenotypes of *NFIA*, *NFIB*, and *NFIX* haploinsufficiencies are emerging and seem to also be reflected in the mouse models, they need corroboration by studies on larger cohorts, particularly in the case of *NFIA* and *NFIB* haploinsufficiency.

The skeletal involvement in Marshall–Smith syndrome is remarkably stronger than in the other phenotypes. The specific N-terminal mutations in this syndrome are thought to function as dominant negative mutations as their coded proteins will compete with wild-type NFI proteins for binding, but are unable to regulate transcription. In this way, they represent a more severe defect of NFIX than haploinsufficiency. Hence, the Marshall–Smith syndrome skeletal phenotype might better match the *Nfix* homozygous knockout mouse phenotype, as already indicated by Malan *et al* (Malan et al., 2010). This could point toward a convergence of human NFI dominant-negative phenotypes with the respective homozygous knockout mouse models. In analogy to *NFIX*, it is also possible that similar mutations exist for *NFIA* and *NFIB* that lead to dominant negatively-acting proteins, with intact DNA binding capacities, but impaired interaction with transcriptional regulators. As both 5' and 3' terminal truncated isoforms of *NFIB* exist in human cells with a proven dominant negative function *in vitro* (Chen et al., 2014; Liu, Bernard, & Apt, 1997), these mutations could occur in introns to alter splicing, and therefore might not be revealed using exome sequencing. If such dominant negative mutations in *NFIA* and *NFIB* exist, one might expect them to be associated with stronger urinary tract and lung defects, respectively, which could potentially be perinatally lethal.

In line with their general role in regulating progenitor cells differentiation, NFI family members have been implicated in cancers in tissues matching their developmental expression (Chen, Lim, et al., 2017). As would be expected, *NFIA*, *NFIB*, and *NFIX* are mainly implicated in brain tumors (Bleeker, Hopman, & Hennekam, 2014). Insertional mutagenesis mouse models for high-grade glioma and medulloblastoma have all identified insertions in the *Nfi* alleles, indicating that disruption of these genes is beneficial for tumor development (Bender et al., 2010; Genovesi et al., 2013; Lastowska et al., 2013; Vyazunova et al., 2014). Furthermore, in a medulloblastoma mouse model, the tumor frequency increased from 38 to 62%, and tumor latency was reduced when one copy of *Nfia* was deleted (Genovesi et al., 2013). Therefore, germline *NFI* haploinsufficiency might increase the development or progression of brain tumors, although this increased risk is likely to be marginal as brain tumors have not been observed in any of the heterozygous knockout mice in almost two decades of breeding. The COSMIC database (https://cancer.sanger.ac.uk/cosmic/) suggests that somatic sequence variants of *NFIA*, *NFIB*, and *NFIX* occur at low frequency in a broad spectrum of cancers. The observed variant distribution is compatible with loss of function and there is some overlap between the variants in tumors and the germline variants reported in the developmental syndromes that are reviewed here. However, copy number variations and structural aberrations, such as translocations, as well as overexpression of NFI genes have also been observed, thus suggesting that their role in oncogenesis is complex and may include tumor suppressive and oncogenic functions depending on the type of tumor (Chen, Lim, et al., 2017). Together, these aspects raise the possibility that NFI-associated disorders, similar to many other overgrowth conditions, may be associated with increased tumor risk. On the basis of current knowledge, however, the tumor risk appears to be low, as only a single patient with (molecularly unconfirmed) Marshall–Smith syndrome has been reported with a Wilms tumor (Ng et al., 2007), with no instance of a malignant disorder being reported for any of the diseases caused by the other NFIs. Based on current knowledge, routine preventive measures in individuals affected by NFI-related disorders are not justified.

5 | CONCLUSIONS

Pathogenic variants in the three *NFI* genes for which such variants have been reported cause an overlapping phenotype characterized by intellectual disability and macrocephaly, except in the case of specific *NFIX* variants which cause a more severe cognitive impairment and marked bone dysplasia, known as Marshall–Smith syndrome. A spectrum of other anomalies can be present which are NFI-gene-specific. The human phenotypes are recapitulated in the various existing mouse models.

The mutation mechanisms are similar in the various *NFI* genes: truncating variants and whole gene deletions act through loss-of-function, and missense variants affect critical residues in the DNA binding domains that cause loss-of-binding and, subsequently, loss-of-function. Other variants that act in a dominant negative manner have only been described in *NFIX* mutations and cause the different phenotypes of Marshall–Smith syndrome.

Variants in *NFI* genes should be considered in every individual with intellectual disability and brain overgrowth, and can be differentiated from one another by additional signs and symptoms. While the diagnosis of Marshall–Smith syndrome can be made on a clinical basis and confirmed by targeted genetic testing, the clinical diagnosis of disorders caused by *NFIA*, *NFIB*, and *NFIX* haploinsufficiency remains challenging due to the lack of high specificity of the observed phenotypes and to the abundance of differential diagnoses (as outlined in this Issue). Hence, we recommend that any broad genetic testing strategy for individuals with unspecified intellectual disability—based on multigene panel, whole exome, or whole genome analysis—should include sequence as well as copy number analysis of *NFI* genes, especially in the presence of macrocephaly. Further studies are needed to determine the influence of the combination of NFI protein functions on phenotypes and to delineate the complete phenotype spectrum, as the presently known number of affected individuals is limited, especially for *NFIA* and *NFIB* variants.

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The authors declare no competing interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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