

BROAD MULTI-GENE PANEL OR WHOLE EXOME SEQUENCING IN MALFORMED FETUSES REVEALS EIGHT DEFINITIVE AND ONE LIKELY DIAGNOSES IN FIFTEEN STUDIED FETUS, IN PRENATAL SETTING

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BACKGROUND

Recently, broad multi-gene panel or whole exome sequencing by Next-generation Sequencing (NGS) became available in the clinical practice at our country. Here we present the results from the first fifteen fetus to which exome sequencing was offered in two prenatal diagnosis centers and assessed in the same laboratory.

METHODOLOGY

After diagnosis of sonographic abnormality and medical termination of pregnancy (13), or foetal death with multiple anomalies confirmed at autopsy (2), a predefined panel of 4813 genes (13) or a trio whole exome sequencing (WES) (2) were performed, following a previous extensive evaluation.

RESULTS AND DISCUSSION

A definitive diagnosis was achieved in eight cases and a likely diagnosis in one (Table 1). The very high diagnostic yield achieved likely derives from cohort ascertainment: recurrence was present at five of the nine families (Figure 1) and very severe, but viable, phenotype, in other two. After genetic diagnosis we found a high recurrence risk in six of the nine families: five with autosomal recessive (AR) diseases and one X-linked (XLR) (Figure 2) – with 25% recurrence risk for future pregnancies and 50% for male pregnancies, respectively. From these six families, four had another pregnancy after diagnosis: to three of them was offered prenatal diagnosis (PND) and the other chose to resort to IVF with egg & sperm donor (Table1).

The likely diagnosis of Fanconi anemia was based on clinical findings. Though we found two potentially pathogenic variants in genes that cause Fanconi anemia disorders (*FANCC* and *FANCD2*), they are both of AR inheritance (Fanconi anemia, complementation group C [MIM 227645] and Fanconi anemia, complementation group D2 [MIM 227646], respectively). A digenic inheritance of Fanconi anemia has not been described to date, however it is known that the *FANCC* gene product interacts in a multiprotein complex whose main function is the post-translational activation of the *FANCD2* gene (Hartmann et al., Am J Hum Genet., 2010).

In the six couples without a diagnosis, we observed recurrence in five (Figure 1). From these recurrent cases, we suspect of: non-monogenic disease in two of them [one with visceral heterotaxy, an highly recurrent disorder even if with unknown etiology; and one with polymalformed fetus in all three pregnancies]; intra-uterine lethality with unknown gene in other two [one with pancreatic agenesis and one with isolated bilateral foot and neonatal death in both fetus of a dizygotic twin pregnancy]; and an AR disease in the remaining one [in which we identified one mutation in a gene that causes an AR disease compatible with the phenotype, but failed to detect a second mutation].

At last, from the fifteen cases in study we found unsolicited findings in three (Table 1). In one of them the mutations responsible for the unsolicited finding were also the causal mutations – *RYR1* gene, causes not only Minicore myopathy (MIM 255320), compatible with the observed phenotype, but also Malignant hyperthermia susceptibility 1 (MIM 145600). In other case, we found a paternally inherited *RAF1* gene mutation associated with Dilated cardiomyopathy 1NN (MIM 615916), posible compatible with the mild cardiopathic signs present in the father. The mutation at *MYBPC3* was described in patients with hypertrophic cardiomyopathy, evaluation of the couple is pending.

	OBSERVED RECURRENCE	MAJOR CLINICAL SIGNS	RESULT	DISEASE (OMIM)	INHER. /RISK	UNSOLICITED FINDINGS	FOLLOW. PREGN.
DEFINITIVE DIAGNOSIS (8)	Recurrent	Fetal hydrops	<i>RAPSN</i> :c.1029_1045del (p.Glu344Cysfs*127)hmtz mat.pat	Fetal akinesia deformation sequence (MIM 208150)	AR (25%)	No	Yes, with normal PND
	Recurrent	Pulmonary hypoplasia	<i>RYR1</i> : c.9262G>A (p.Val3088Met) hmtz mat <i>RYR1</i> : c.13639G>A (p.Val4547Met) hmtz pat	Minicore myopathy (MIM 255320)	AR (25%)	<i>RYR1</i> : Malignant hyperthermia susceptibility 1 (MIM 145600)	Yes, with normal PND
	Isolated	Microcephaly; cerebellar hypoplasia	<i>PNKP</i> : c.1549C>T (p.Gln517*) hmtz mat <i>PNKP</i> : c.1027+2T>C hmtz pat	Microcephaly, seizures, and develop. delay (MIM 613402)	AR (25%)	<i>MYBPC3</i> : c.2827C>T (p.Arg943*) hmtz Cardiomyopathy, hypertrophic(MIM 115197)	Yes, with normal PND
	Isolated	Ventriculomegaly	<i>L1CAM</i> : c.1267+1G>A hemizigosis; mat	Hydrocephalus due to aqueductal stenosis (MIM 307000)	XLR (50% male)	No	No
	Recurrent	Lissencephaly	<i>PAFAH1B1</i> : c.162dup [p.(Trp55Metfs*6) hmtz dn?	Lissencephaly-1 (MIM 607432)	AD (1%)	No	No
	Isolated	Polymalformative syndrome	<i>MKS1</i> : c.417G>A hmtz	Meckel syndrome 1 (MIM 249000)	AR (25%)	No	No
	Isolated	Cystic hygroma	<i>SHOC2</i> : c.4A>G (p.Ser2Gly) hmtz dn	Noonan-like syndrome with loose anagen hair (MIM 607721)	AD (1%)	<i>RAF1</i> : c.1502A>G (p.Gln501Arg) hmtz pat Cardiomyopathy, dilated, 1NN (MIM 615916)	No
	Recurrent	Lissencephaly; ventriculomegaly	<i>ASPM</i> :c.7747_7748del (p.Ile2583thrs*5) hmtz pat <i>ASPM</i> :c.593T>A (p.Leu198*) hmtz mat	Microcephaly 5, primary, autosomal recessive (MIM 608716)	AR (25%)	No	Yes, with egg and sperm donation
LIKELY DIAGNOSIS (1)	Recurrent	Renal agenesis; cardiopathy	<i>FANCC</i> : c.668T>C (p.Val223Ala) hmtz pat <i>FANCD2</i> : c.4137T>A (p.Asn1379Lys) hmtz mat	Fanconi anemia	?	No	No
WITHOUT DIAGNOSIS (6)	Recurrent: 5 Isolated: 1	Severe (6)	-	-	-	No (6)	?

Table 1. Sample description and detailed results. The clinical indication, obstetric history and results are detailed per case only for those in which a diagnosis was achieved.

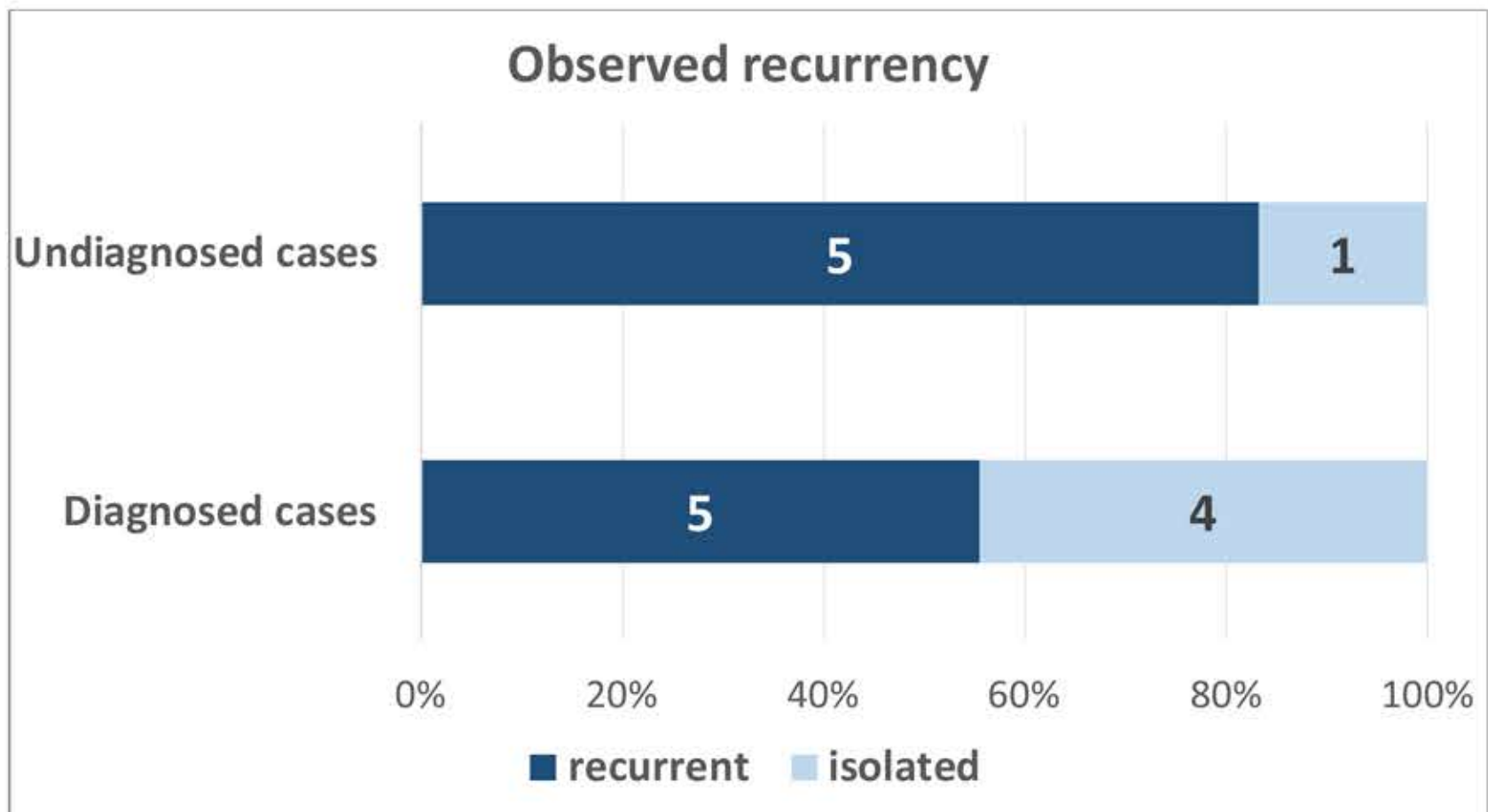


Figure 1. Observed recurrency in diagnosed and undiagnosed cases.

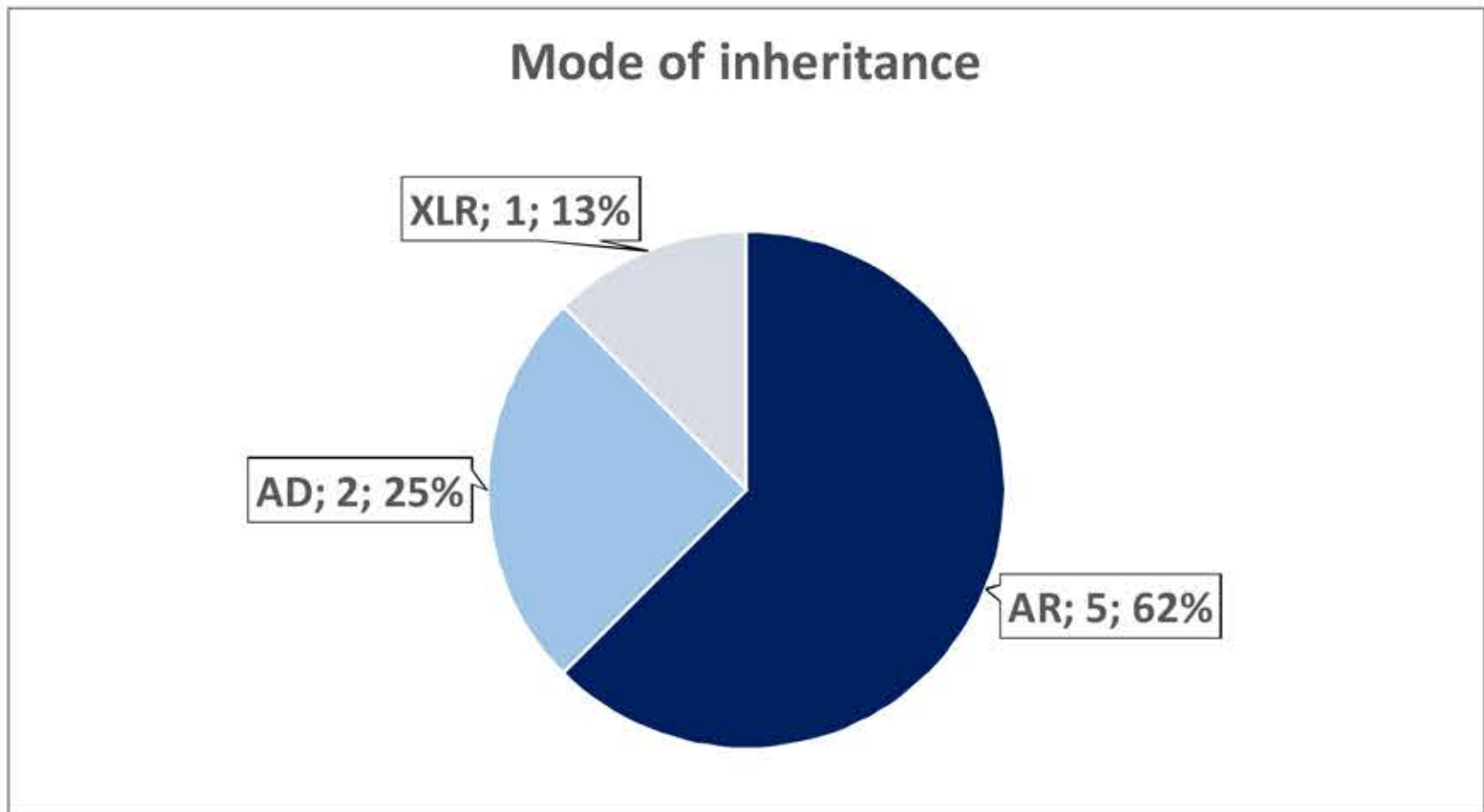


Figure 2. Mode of inheritance of the diseases found in the definitive diagnosed cases [Mode of inheritance, n, %].

CONCLUSION

At the moment, our proposal is to restrain the use of clinical exome or WES to cases with recurrence or high severity and only if collaboration with a medical geneticist is assured. In a short future, the use of these tests will increase and become part of the initial diagnostic approach.



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