The Frequency and Impact of Dependent Alleles in Expanded Carrier Screening

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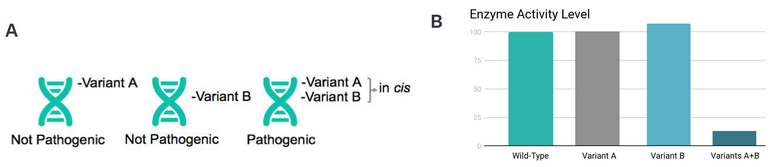
Introduction

Variants whose pathogenicity is contingent on the presence or absence of a second variant in the same gene (i.e., "dependent alleles") present unique challenges for variant curation, results reporting, and genetic counseling. The classification of such alleles depends on the context in which the variant is identified (see Figure 1). For carrier screening, the presence of a dependent allele may greatly affect an individual's reproductive risk. However, because of the complex nature of dependent alleles, routine classification protocols and reporting structures may not accurately reflect the risk associated with these alleles, particularly those with a high population frequency that may easily be misclassified as benign. Specific curation techniques and reporting are required to provide an accurate risk assessment and appropriate genetic counseling.

Figure 1. Example of a Dependent Allele



In this example, the combination of variant A and variant B is significantly associated with a given condition (Figure 1A). When present alone, variant A does not significantly affect protein function and is not considered pathogenic. However, when this variant is present in cis with variant B, enzyme activity is dramatically reduced (Figure 1B). Based on this evidence and the association between the two variants in patients, the combination of variant A and variant B is considered pathogenic and any carrier of this allele would have an increased risk for having an affected child.

Methods

We analyzed the results from more than 206,000 individuals who underwent expanded carrier screening at Counsyl. Dependent alleles were identified by variant curation when published data showed that a specific combination of variants was significantly associated with the disease and that the allelic interaction significantly affected protein function (Table 1). The extensively studied CFTR p.R117H and 5T variants were excluded from the analysis.

Table 1: Dependent Alleles Identified by Expanded Carrier Screening at Counsyl

Gene	Condition	Dependent Allele Description	Reference(s)
BTD	Biotinidase deficiency	p.D444H + p.A171T in cis = pathogenic (severe BTD allele[a])	1, 2
BTD	Biotinidase deficiency	p.D444H + p.F403V in cis = pathogenic (severe BTD allele[a])	1, 2
MEFV	Familial Mediterranean fever	p.F479L + p.E167D in cis = pathogenic	3, 4
NPHS2	Steroid-resistant nephrotic syndrome	p.R229Q + C-terminal missense variants[b] in trans = pathogenic p.R229Q in combination with other trans alleles = variant of uncertain significance	5, 6
GALC	Krabbe Disease	p.I82M + p.I305V in cis = pathogenic	7
PKHD1	Autosomal recessive polycystic kidney disease	p.I3177T + p.P805L in cis = pathogenic	8, 9

[a] p.D444H in the absence of p.A171T or p.F403V is associated with partial biotinidase deficiency.
[b] Including: c.851C>T, p.A284V; c.871C>T, p.R291W; c.890C>T, p.A297V; c.928G>A, p.E310K; c.983A>G, p.Q328R; c.862G>A, p.A288T.

Results

Frequency

The frequencies of dependent alleles among individuals screened at Counsyl were comparable to the frequencies found in ExAC (Table 2). The most frequent dependent alleles were BTD p.D444H (allele frequency 3.15%), NPHS2 p.R229Q (allele frequency 2.84%), and GALC p.I305V (allele frequency 1.02%) (Table 2, gold). Independently, these variants are not considered pathogenic, as they have a high population frequency and homozygotes are typically asymptomatic. The overall frequency of additional dependent alleles ranged from 0.00024% to 0.032%, with allele frequencies varying by condition and ethnicity (Table 2).

Table 2: Frequency of Dependent Alleles

Gene	Allele	Counsyl Allele Frequency & Population with Highest Frequency	ExAC Allele Frequency & Population with Highest Frequency
BTD	p.D444H	3.15% (12991/412480) NEU 3.75% (3.681 - 3.827% 9798/261016)	3.17% (3844/121398) FIN 5.40% (4.88-5.97% 357/6614)
BTD	p.A171T	0.032% (130/409432) FRC 0.325% (0.089 - 0.829% 4/1232)	0.04% (47/120592) NFE 0.07% (0.05-0.09% 44/66336)
BTD	p.F403V	0.00024% (1/412572) HIS 0.003% (0 - 0.019% 1/29668)	Not found in ExAC
MEFV	p.F479L	0.0065% (27/412486) NEU 0.007% (0.004 - 0.011% 19/261012)	0.003% (4/121412) NFE 0.01% (0.00-0.02% 4/66740)
MEFV	p.E167D	0.006% (26/412270) NEU 0.007% (0.004 - 0.011% 19/261888)	0.003% (1/29174) NFE 0.01% (0.00-0.04% 1/13236)
NPHS2	p.R229Q	2.84% (11694/412396) AJ 4.502% (4.278 - 4.735% 1446/32118)	3.17% (3844/121398) FIN 5.40% (4.88-5.97% 357/6614)
NPHS2	p.A284V	0.004% (17/412336) HIS 0.044% (0.023 - 0.075% 13/29652)	0.04% (47/120592) NFE 0.07% (0.05-0.09% 44/66336)
NPHS2	p.R291W	0.0012% (5/412320) EAS 0.009% (0.001 - 0.031% 2/23354)	Not found in ExAC
NPHS2	p.A297V	0.00048% (2/412546) AFR 0.008% (0.001 - 0.029% 2/25130)	0.003% (4/121412) NFE 0.01% (0.00-0.02% 4/66740)
NPHS2	p.E310K	0.00024% (1/412578) HIS 0.003% (0 - 0.019% 1/29668)	0.003% (1/29174) NFE 0.01% (0.00-0.04% 1/13236)
NPHS2	p.Q328R	0.00024% (1/412536) NEU 0.00038% (0 - 0.002% 1/262040)	Not found in ExAC
GALC	p.I82M	0.0002% (1/412536) EAS 0.004% (0.0001 - 0.02% 1/23342)	Not found in ExAC
GALC	p.I305V	1.02% (4185/412198) EAS 10.1% (9.7 - 10.4% 2346/23332)	0.008% (963/119748) EAS 9.85% (9.23-10.50% 841/8540)
PKHD1	p.I3177T	0.004% (22/497402) NEU 0.007% (0.004 - 0.01% 21/316668)	0.0041% (5/121294) NFE 0.007% (0.002 - 0.017% 5/66720)
PKHD1	p.P805L	0.006% (25/417092) NEU 0.007% (0.005 - 0.011% 23/316678)	0.0058% (7/121380) NEU 0.01% (0.004 - 0.022% 7/66724)

Gold = Alleles with the highest frequency in this cohort. These variants have a frequency that would result in a classification of likely benign or benign. NEU: Northern European; FIN: Finnish; NFE: Non-Finnish European; FRC: French Canadian/Cajun; HIS: Hispanic; AJ: Ashkenazi Jewish; EAS: East Asian; AFR: African.

Co-Occurrence

A total of 128 individuals (0.062%) were found to carry both BTD p.D444H and p.A171T (Table 3). These individuals would have an increased risk for having a child with biotinidase deficiency if their partner also carries a pathogenic BTD variant. For NPHS2 p.R229Q, a carrier is only at risk for having a child with steroid-resistant nephrotic syndrome when his/her partner has one of certain pathogenic C-terminal missense variants.5 NPHS2 p.R229Q was found in 3.3%, 4.5%, and 7.0% of Northern European, Ashkenazi Jewish, and Finnish patients, respectively, although no partners were found to carry relevant C-terminal NPHS2 variants (Table 3). Co-occurrence frequencies of the identified dependent alleles are summarized in Table 3. The precise frequency of dependent allele co-occurrence among all tested individuals could not be calculated because the total number of individuals tested varied by allele. However, based on the data, it may be estimated that co-occurrence was found in approximately 0.08% of the cohort (CFTR p.R117H and 5T variants excluded).

Reporting

To accurately reflect the residual risk for disease, patient reports require additional information concerning the variant's classification and when the variant may cause disease. In addition, genetic counseling for dependent alleles is complicated by the fact that their associated risks depend on the context in which they are identified. Examples of the additional information included on patient reports are summarized in Table 3, while Figure 2 shows a sample of a patient report for an individual with both BTD p.D444H and BTD p.A171T.

Study Limitations

The main limitation of this study was the inability to confirm phase in individuals with co-occurrence of the identified dependent alleles. Consequently, the frequency of co-occurrence may have been overestimated for those conditions in which the two variants needed to be present in cis.

Figure 2: Sample Report for an Individual with a Dependent Allele

POSITIVE: CARRIER

Biotinidase Deficiency

Gene: BTD | **Inheritance Pattern:** Autosomal Recessive

Reproductive risk: 1 in 62
Risk before testing: 1 in 13,000

Patient	[redacted]	**No partner tested**
Result	Carrier	N/A
Variant(s)	NM_000060.2(BTD):c.1330G>C(D444H) heterozygote NM_000060.2(BTD):c.511G>A(A171T) heterozygote	N/A
Methodology	Sequencing with copy number analysis	N/A
Interpretation	This individual is a carrier of biotinidase deficiency. Carriers generally do not experience symptoms. A171T has only been observed in combination with D444H in the literature which indicates they are likely to be on the same chromosome. When A171T and D444H are present on the same chromosome, this is equivalent to a profound biotinidase deficiency mutation. D444H is a partial biotinidase deficiency mutation.	N/A

Table 3: Frequency of Co-Occurrence and Reporting for Dependent Alleles

Gene	Condition	Frequency of Co-Occurrence[a]	Reporting (Additional Information on Patient Reports)
BTD p.D444H + p.A171T in cis	Biotinidase deficiency	0.062% (128/206173) FRC 0.649% (0.177 - 1.654% 4/616)	**p.D444H**: "D444H is a partial biotinidase deficiency mutation." **p.A171T**: "A171T has only been observed in combination with D444H in the literature which indicates they are likely to be on the same chromosome. When A171T and D444H are present on the same chromosome, this is equivalent to a profound biotinidase deficiency mutation."
BTD p.D444H + p.F403V in cis	Biotinidase deficiency	0.00048% (1/206222) HIS 0.007% (0 - 0.038% 1/14832)	**p.F403V**: "F403V has only been observed in combination with D444H in the literature which indicates they are likely to be on the same chromosome. When F403V and D444H are present on the same chromosome, this is equivalent to a profound biotinidase deficiency mutation."
MEFV p.F479L + p.E167D in cis	Familial Mediterranean fever	0.012% (25/206098) ME 0.09% (0.018 - 0.262% 3/3347)	**p.F479L**: "In isolation, the pathogenicity of F479L is unknown. When F479L and E167D are present on the same chromosome, this is associated with familial Mediterranean fever." **p.E167D**: "In isolation, the pathogenicity of E167D is unknown. When E167D is present on the same chromosome as another variant, it may be associated with familial Mediterranean fever."
NPHS2 p.R229Q + C-terminal variants in trans[b]	Steroid-resistant nephrotic syndrome	0%[c]	**p.R229Q**: "The pathogenicity of R229Q is dependent on the variant observed on the other chromosome. There is insufficient evidence that individuals who are homozygous for this variant are at risk for steroid-resistant nephrotic syndrome."
GALC p.I82M + p.I305V in cis	Krabbe Disease	0.0005% (1/205970) EAS 0.009% (0 - 0.048% 1/11663)	**p.I82M**: Rare. Evaluated on a per case basis. **p.I305V**: Classified as benign when present alone.
PKHD1 p.I3177T + p.P805L in cis	Autosomal recessive polycystic kidney disease	0.0076% (19/248612) NEU 0.011% (0.007 - 0.018% 18/158281)	**p.I3177T**: In isolation, the pathogenicity of I3177T is unknown. When I3177T and P805L are present on the same chromosome, this is associated with autosomal recessive polycystic kidney disease. **p.P805L**: In isolation, the pathogenicity of P805L is unknown. When P805L and I3177T are present on the same chromosome, this is associated with autosomal recessive polycystic kidney disease.

Gold = Most commonly found dependent allele combination in this cohort. FRC: French Canadian/Cajun; HIS: Hispanic; ME: Middle Eastern; EAS: East Asian; NEU: Northern European.
[a] For BTD, MEFV, and GALC dependent alleles, phase of the 2 variants could not be confirmed.
[b] Including: c.851C>T, p.A284V; c.871C>T, p.R291W; c.890C>T, p.A297V; c.928G>A, p.E310K; c.983A>G, p.Q328R; c.862G>A, p.A288T.
[c] No partner was found to carry a relevant C-terminal missense variant. The number of partners ranged from 22,147 to 22,161, depending on the allele.

Conclusions

In isolation, certain dependent alleles could be misreported as having little or no reproductive risk, particularly those with a high population frequency. However, an advanced workflow that takes into consideration the consistent association between two variants (in cis or in trans) and the functional significance of this association allows for a more accurate classification. Laboratories must be diligent in the handling of dependent alleles observed in carrier screening to ensure appropriate patient counseling and management. Awareness and continued study of dependent alleles are essential for ensuring accurate variant classification, comprehensive reporting, and appropriate genetic counseling.

REFERENCES: **1.** Norrgard et al., Hum Mutat.1998;11(5):410. **2.** Swango et al., Hum Genet. 1998;102(5):571-5. **3.** Rossou et al., Clin Chem. 2005;51(9):1725-7. **4.** Bonyadi et al., Clin Genet. 2009;76(5):477-80. **5.** Tory et al., Nat Genet. 2014;46(3):299-304. **6.** Phelan et al., Clin Kidney J. 2015;8(5):538-42.