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REVIEW

Digenic inheritance and genetic modifiers

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Digenic inheritance (DI) concerns pathologies with the simplest form of multigenic etiology, implicating more than 1 gene (and perhaps the environment). True DI is when biallelic or even triallelic mutations in 2 distinct genes, in *cis* or in *trans*, are necessary and sufficient to cause pathology with a defined diagnosis. In true DI, a heterozygous mutation in each of 2 genes alone is not associated with a recognizable phenotype. Well-documented diseases with true DI are so far rare and follow non-Mendelian inheritance. DI is also encountered when by serendipity, pathogenic mutations responsible for 2 distinct disease entities are co-inherited, leading to a mixed phenotype. Also, we can consider many true monogenic Mendelian conditions, which show impressively broad spectrum of phenotypes due to pseudo-DI, as a result of co-inheriting genetic modifiers (GMs). I am herewith reviewing examples of GM and embark on presenting some recent notable examples of true DI, with wider discussion of the literature. Undeniably, the advent of high throughput sequencing is bound to unravel more patients suffering with true DI conditions and elucidate many important GM, thus impacting precision medicine.

KEYWORDS

co-inheritance, digenic inheritance, DNA variants, genetic alpha effect, genetic modifiers, high throughput sequencing, phenotypic heterogeneity, polymorphisms, pseudo-digenic inheritance

1 | INTRODUCTION

The advent of next generation sequencing (NGS) technologies during the past decade, inescapably leads to finding variants we would not have found previously, which may or may not have significance for, or impact, the phenotype. This realization makes it very important to apply measures of discriminating the pathogenic from the neutral variants. Based on several measures of evaluating the pathogenicity of DNA variants they are classified as clearly or likely to be pathogenic, of unknown significance, unlikely to be pathogenic or clearly not pathogenic.[1] It is worth noting that between the concept of a clearly pathogenic and a clearly not pathogenic DNA variant there is an abyss of variants of variable functional significance. Things become more complicated when we implicate not 1 but 2 or more DNA variants to describe the symptoms and justify the diagnosis; which is where digenic inheritance (DI) and genetic modifier (GM) come into play.

One particular class of interesting mutations is those described as hypomorphic. Hypomorphic mutations (one type of Muller's Morphs, after Nobel laureate Hermann J. Muller), lead to reduced gene activity (as opposed to hypermorphic mutations, which lead to increased gene activity). They refer to clearly mutant DNA variants which retain some residual activity. Depending on several factors and the specific gene at fault, 2 or more such mutations are required to be co-inherited in *cis* (on same chromosome and genetically linked) or in *trans* (on different alleles of same gene or on 2 different genes) in order to produce a recognizable phenotype and such mutations may account for cases with incomplete penetrance. They are clearly different from recessive mutations which can be severe but yet insufficient to confer a phenotype because the 1 normal dose from the wild-type allele is sufficient to maintain homeostasis and health. It is frequent, for example, to have severe, even non-sense mutations with total loss-of-function, especially in enzymes, that act as recessive in healthy carriers.

Speaking of clearly pathogenic mutations in monogenic disorders, 1 genetic defect may be responsible for the expression of a disease phenotype, which describes a defined diagnosis. When concentrating on the mutated gene, the full spectrum and/or the severity and/or the age-at-onset of the disease may be determined by several factors:

1. The specific mutated gene (consider genetic heterogeneity).
2. The position of the mutation in the protein (ie, specific functional domain, N-terminal or C-terminal).
3. The nature of the mutation, that being a single nucleotide substitution, a small indel or a large deletion/insertion, a termination codon, a splice site defect or a frameshift.
4. In cases of single aminoacid substitutions, the actual nature of the substitution, in terms of the size and the biochemistry of the involved aminoacid side chains (charged vs not-charged and hydrophilic vs hydrophobic).[2] This also applies to DI as shown below.

2 | DIGENIC INHERITANCE AND GENETIC MODIFIERS

I can think of 3 distinct examples of DI:

1. True DI, strictly speaking non-Mendelian, as the simplest form of oligogenic inheritance. An important element is that the patient will only manifest the disease when 2 non-allelic mutations, on separate genes are co-inherited, as necessary and sufficient to elicit the phenotype. A patient with steroid-resistant focal segmental glomerulosclerosis (FSGS), had inherited 2 heterozygous mutations, 1 each in the podocin and nephrin genes.[3]
2. Inheritance of a single primary mutation that establishes the diagnosis and a second DNA variant, which modifies the phenotype, exacerbating the clinical picture, under a pseudo-DI scenario. One expects a broad phenotypic spectrum depending on the gene(s) at fault. The putative GM is only causative when co-inherited on the background of a primary "driver" mutation. When introducing the concept of GM, we accept there is no limit to the number of such modifiers that might have a perceptible contribution to the phenotype. One could envision that a single major modifier is adequate to discernibly accentuate the clinical presentation or multiple ones alike, with separate or synergistic influence. Consider a variant in the 5′ end of TGFbeta1 that modifies lung disease in cystic fibrosis (CF).[4]
3. Coincidental independent segregation of 2 separate disease entities, each one caused by mutations in separate linked or unlinked genes/loci. Each one follows a classic Mendelian mode of inheritance. A rare occurrence is the co-inheritance of polycystic kidney disease and Marfan syndrome.[5]

Sometimes the borders of the scenarios described may be blurred owing to the huge and still largely incomprehensible complexity of the human genome, but I hope the readers agree that true DI should be crystal-clear as the simplest case of oligogenic inheritance not following Mendelism, as defined above. The concept of GM acting as concomitant heritable events in pseudo-DI, offers potentially the most blurred scenario, conditional to their contribution in different disease entities, which admittedly may not always be easy to decipher, owing to their nature as quantitative traits of variable effect size.

In true DI, either of the 2 mutations alone does not lead to a perceptible phenotype. This definition deterministically implies no Mendelism. Perhaps this is where I somewhat differ with the broader definition that Schaffer gave in his elegant review, where in his DI definition he included cases that "can be better explained" by invoking the contribution of 2 variants at different loci, linked or unlinked, than each one on its own.[6] Without being dogmatic, such situations should not be classified as genuine true DI. Rather, they would be better viewed as monogenic and the variable phenotype attributed to the role of modifier(s) or genes adding other symptoms, than to the role of a second gene as necessary and sufficient, in determining whether or not a disease phenotype will manifest (pseudo-DI). I believe most experts would agree that a GM is a DNA variant that exerts an epistatic effect on the phenotype, which is invariably determined by a primary gene. The variable expressivity (clinical or phenotypic heterogeneity), may be confounded by the contribution of one or more GM. This phenotypic heterogeneity, which sometimes can be hugely extensive ranging from very benign to very severe and life threatening, is part of the spectrum of symptoms which pertain to the genotype/phenotype correlation focusing on the primary gene at fault.[7–9] Therefore, the final phenotype in a Mendelian monogenic disorder can be an amalgamation of multiple factors: (1) the actual mutated gene (and the kind and position of the primary decisive mutation), (2) the co-inheritance of GMs that may predispose to a more severe or milder phenotype, (3) somatic mosaicism and other atypical patterns of inheritance,[10] including epigenetic phenomena, and (4) environmental factors which may or may not be known, although amply suspected.

3 | PRIMARY MUTATIONS AND GENETIC MODIFIERS AS EXAMPLES OF PSEUDO-DI

It has been proposed that GM influence the end-deep phenotype of Mendelian disorders, in terms of the full spectrum and/or the severity and the age-at-onset. It is not an innovation on my part to expect that this scenario will prove to be the most prevalent, involving many if not most classical monogenic conditions, characterized by extreme clinical heterogeneity, reminiscent to a phenotypic chameleon. This phenotypic chameleon is evident in inter-familial and even intra-familial heterogeneity, reflected in incomplete penetrance or in severe (or mild) and with early (or late) onset symptoms, depending on the disease.

The hitherto used approach by researchers that witness patients with same diagnosis and similar pathogenic variants, but placed on a broad spectrum of symptoms, is the search for contributory DNA variants in candidate modifier genes. These could be the second or third gene that is responsible for the same monogenic disease, owing to known genetic heterogeneity. Excellent examples are the long QT syndrome and Bardet-Biedl syndromes, each caused by mutations in one of more than a dozen genes, Alport syndrome (AS) with 3 genes (*COL4A3*/*COL4A4*/*COL4A5*)[11]; cystinuria (*SLC3A1*/*SLC7A9*)[12]; polycystic kidney disease (*PKD1*/*PKD2*)[13]; inherited cardiomyopathies with mutations in 8 sarcomere genes accounting for only 60% of cases[14] and numerous others. Equally good candidates are genes encoding proteins partaking in the same protein complex (trimeric collagen genes, multimeric receptors), or genes coding for interacting partners, or for proteins the function of which converges to the same

pathogenetic pathway or participate in higher structures (eg, glomerular slit diaphragm). Epistatic genes that may affect rates of transcription or mRNA stability, in the form of epigenetic interference of miRNAs, cannot be excluded.

A prime example of the role of GM in monogenic glomerulopathies, is thin basement membrane nephropathy (TBMN) when it is caused by heterozygous *COL4A3* or *COL4A4* mutations. These patients are actually the carriers of the autosomal-recessive form of AS (ARAS), who are not healthy but instead they present with autosomal-dominant familial microscopic hematuria (MH). This condition was formerly known as familial benign hematuria. However, voluminous data documented that although many patients will stay for life with isolated MH, others will exhibit a progressive glomerulonephritis and FSGS, with proteinuria and kidney function decline, even end-stage renal disease (ESRD) later in life (average age 56 years).[15] We refer to this condition as later-onset Alport-related nephropathy while several other authors name it autosomal-dominant AS, in most cases without extra renal features.[16,17] We and others hypothesized that the co-inheritance of GM might account for the long-term predisposition of a subset of patients to severe or mild disease.

Our thesis is that on long follow-up, the full phenotypic spectrum of patients presenting with MH, behaves as a multifactorial condition, implicating primary genes, modifier genes and environmental factors. We published on 2 DNA variants in the *NPHS2* (podocin) gene (p.Arg229Gln, p.Glu237Gln), the product of which is part of the slit diaphragm of the glomerular filtration barrier, interacting with nephrin (Figure 1). Mutation *NPHS2*-p.Arg229Gln was previously linked to steroid-resistant nephrotic syndrome, a highly heterogeneous autosomal-recessive nephropathy. In functional cell culture experiments, the alternative variant proteins impaired the interaction with other slit diaphragm partners and interfered with normal trafficking, demonstrating perinuclear staining.[18] More recently we reported on the putative predisposing role of a variant in the *NEPH3* gene (filtrin), also a component of the podocyte slit diaphragm.[19] Patients carrying heterozygous *COL4A* mutations and co-inheriting the variant *NEPH3*-p.Val353Met had an increased risk to progressive kidney failure. Further work with undifferentiated podocytes showed disturbance of variant p.353Met homo-dimerization and hetero-dimerization with nephrin, while p.353Met elicited the activation of the unfolded protein response pathway when overexpressed in stressed cultured cells, thus attesting to its functional significance. Importantly, genetic epidemiology studies combining the general population cohorts of Framingham, KORAF4 and SAPHIR studies (11 258 individuals), revealed significant association with microalbuminuria in homozygous subjects[19] (Figure 2).

In another work we reported on the epistatic role of *MYH9/APOL1* region on familial hematuria genes. Exploiting several cohorts of patients with familial hematuria as a common finding, we showed association of "Severe" disease in CFHR5 nephropathy (a form of C3 glomerulopathy) with *MYH9* variant rs11089788 that we confirmed in an independent cohort. Previous genome-wide association studies have identified variants in the *MYH9* and its closely linked *APOL1* gene to confer major susceptibility towards ESRD in various types of renal diseases.[20]

In the same Cypriot CFHR5 nephropathy cohort, we presented evidence for yet another putative modifier.[21] Specifically, a variant in the target site of miR-1207-5p in the 3′ UTR of *HBEGF* was associated with severity of disease. *HBEGF* (heparin binding epidermal

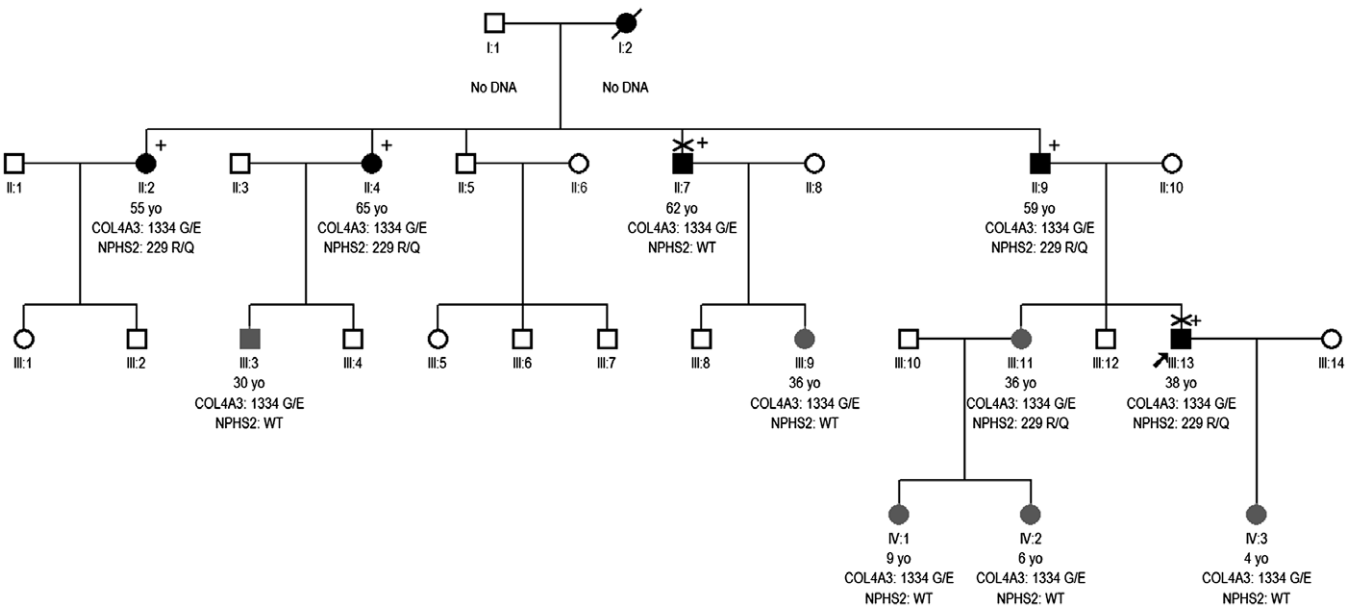

FIGURE 1 In family CY5376, *NPHS2* variant p.Arg229Gln segregates with severe phenotype, on the background of collagen-IV nephropathy and thin basement membrane nephropathy (TBMN) due to inheritance of mutation *COL4A3*-p.Gly1334Glu (COL4A3:1334G/E). Black symbols: patients heterozygous for the mutation; (+) symbol: severe phenotype. Patient II-7 also has a severe phenotype, which is attributed to his co-occurrence of TBMN and vesicoureteral reflux (VUR). Some other patients are heterozygous for the risk variant p.Arg229Gln and have mild disease but are still very young and consequently cannot be classified as "Mild" or "Severe" (filled grey symbols). The 2 patients marked with an (×) symbol had exhibited VUR in childhood. WT: normal; *COL4A3*:1334G/E: heterozygous for *COL4A3*-p.Gly1334Gln; *NPHS2*: 229R/Q, heterozygous for *NPHS2*-p.Arg229Gln (reproduced with permission from Reference[18])

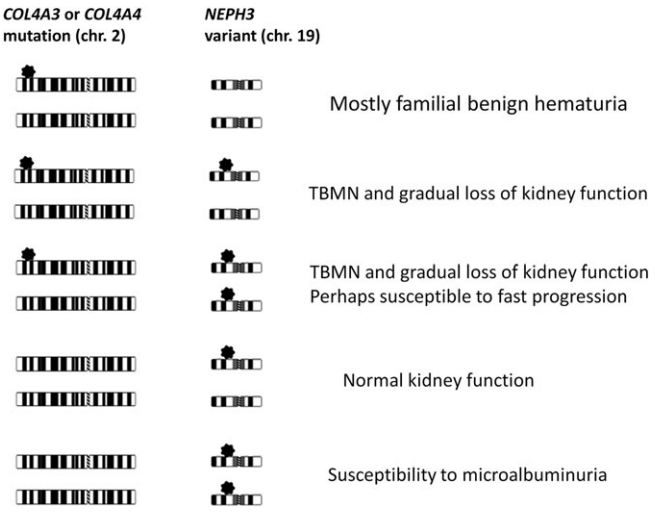

FIGURE 2 A hypothetical model to explain the severe disease phenotype of patients with heterozygous *COL4A3/COL4A4* mutations who develop later-onset Alport-related nephropathy. When searching for modifiers amongst genes expressed in the renal glomerulus, we identified variant *NEPH3*-p.Val353Met. In addition to its contribution by increasing the risk to severe disease on statistical grounds, functional studies corroborate its role. The alternative allele interferes with its homodimerization and heterodimerization with nephrin, the most important component of the slit diaphragm, part of the glomerular filtration barrier. The variant in heterozygosity confers a risk only when co-inherited on the background of a collagen-IV nephropathy; however, in homozygosity on its own may increase susceptibility to albuminuria (reproduced with permission from Reference[19])

growth factor) is expressed in podocytes and plays a role in glomerular physiology.

Notwithstanding the relatively small size of the cohorts we studied, perhaps our success in identifying candidate GM is attributed to the reduced genomic complexity of these cohorts. More than 70% of the TBMN patients carried the same *COL4A3* founder mutation, p.Gly1334Glu, while all CFHR5 nephropathy patients carried the endemic exon 2 to 3 duplication.

Even though our initial hypothesis provided that GM are variants which on their own might be purely neutral, we are prepared to accept that most might represent hypomorphic mutants with residual activity, which exert an effect when found on the background of a primary defect. In a looser definition, the serendipitous co-inheritance of the primary mutation and the modifier can better explain the phenotype. Alternatively, they could be recessive mutations, without recognizable symptoms when inherited singly. One limitation of our investigations is the relatively small number of subjects with the studied phenotype, mainly because of the rarity of the hereditary condition and the relative rarity of the variants (MAF = 2%-3%), thus preventing validation studies on independent cohorts, which are badly needed. Also, in several similar occasions the association with GM does not lead to an absolute relationship. This implies that the effect of the modifier no matter how strong it is, might not be sufficient on its own, therefore more than one may be necessary or the overall situation is more complex. Also plausible is that the full spectrum of some monogenic conditions is the result of oligogenic rather than strict pseudo-DI, or that the environment has substantial contribution.[15,16,22]

A few notable examples of GM, accompanied by a variable level of certainty with regards to their role and actual effect, are discussed in the following sections.

3.1 | Alport syndrome and focal segmental glomerulosclerosis

In a consanguineous family segregating X-linked AS a doubly mutant *COL4A5* allele could explain the phenotype in males, which, however, was of unusually early onset. An infant brother had presented with nephrotic syndrome and progressed to ESRD by the age of 3 years and a young sister by 8 years. This unusual severity, supported by biopsy results of podocyte foot processes fusion, prompted more studies in podocyte-related genes. Two in *cis* variants in homozygosity affected highly conserved aminoacids in the *MYO1E* gene, p.(Lys118Glu) and p.(Thr876Arg).[23] *MYO1E* encodes a podocyte-expressed non-muscle myosin and is a rare cause of familial FSGS and nephrotic syndrome.[24] This case is placed on the borderline between genetic modification and independent segregation of 2 separate disease entities, as either one alone generates a phenotype. The expression of both genes in the glomerulus inescapably renders either one modifier of the other.

3.2 | Autosomal-dominant polycystic kidney disease and DKK3

Severity in autosomal-dominant polycystic kidney disease (ADPKD) is primarily determined by the mutated gene (*PKD1* vs *PKD2*), with the *PKD2* mutations associated with more than a decade later age-at-onset. Nevertheless, GM has been implicated. Perhaps the strongest one, which, however, has not been replicated in a second study, is a variant in the *DKK3* gene that antagonizes the Wnt/β-catenin signaling and thus modulating cyst growth.[25] A recent publication reported that the same protein constitutes an immunosuppressive and a profibrotic epithelial protein that might even serve as a potential therapeutic target and diagnostic marker in renal fibrosis.[26]

3.3 | Ciliopathies

In the genetically heterogeneous group of ciliopathies, patients in a nephronophthisis cohort had a 7-fold increased risk for retinal degeneration if they carried a DNA variant in the *AHI1* gene. *AHI1* encodes a cilium-localized protein and was not the primary gene at fault, therefore, apparently it conferred a strong modifying effect.[27] Another example is a common variant (p.A229T) in the *RPGRIP1L* (retinitis pigmentosa GTPase regulator-interacting protein-1 like), a ciliary gene mutated in Meckel-Gruber (MKS) and Joubert (JBTS) syndromes. This variant conferred a higher risk for retinal degeneration in patients with hereditary ciliopathies due to mutations in other genes.[28] The unusually large repertoire of genes mutated in ciliopathies serves as an exemplar of recessive genes, encoding proteins co-localized in macromolecular structures of prime significance (in this case, the primary cilium of epithelial cells), which are common targets for variants that act as GM. In fact this is a lesson we are learning as

we go ahead; that is, the first likely candidate as GM are peer gene products.

3.4 | Cystic fibrosis

A classical monogenic disease of autosomal-recessive inheritance is CF, the most frequent potentially lethal inherited disorder, with a carrier frequency of 1/25-30 in most Caucasian populations. Similarly, CF is recognized to have a polygenic etiology with regards to its full symptomatology and organ involvement. Many studies that include candidate gene approach and genome-wide analyses have been performed with variable success. Overall, the variability of symptoms in CF patients in the various organs is such that even though the allelic genotype accounts for it to some extent, the contribution of tens of GM is indisputable. Perhaps the most well-accepted and replicated one, is a variant in the transforming growth factor beta-1 (*TGFβ1*), with a well-known role in airway inflammation and remodeling, thus affecting asthma and chronic obstructive pulmonary disease.[29]

With no intention for an exhaustive discussion of GM, we conclude this section by observing that their putative action may relate directly or indirectly to the primary gene's function, in ways that might or might not be obvious. Stemming from experience in kidney disorders, with a hypothesis-driven approach, one can envision the effect of variants in genes co-expressed in the glomerulus and the slit diaphragm (for inherited glomerulopathies) or the complement cascade or the cilium for the many complementopathies or ciliopathies, respectively. However, genes involved in tubulo-interstitial fibrosis, inflammatory processes, autophagy or the unfolded protein response signaling cascade may exert a variable effect. Other approaches, using modern machine learning algorithms and genome-wide searches, shall enable the non-biased identification of variants with small effect, perhaps documented only on statistical grounds. Deep phenotyping, the study of adequately large cohorts and unequivocal functional effect will empower the chances for success.

4 | BILINEAL INHERITANCE OF 1 DISEASE OR CO-INHERITANCE OF 2 DIFFERENT

Even for true monogenic disorders, high throughput sequencing (HTS) has led to the discovery of DI, where in some rare occasions the patients co-inherit 2 separate genic variants, something that also can be described as double or *trans*-heterozygosity. Those genes can be linked or unlinked. In these cases, obviously, we witness DI (not true DI) which has nothing to do with non-Mendelian inheritance as a subset of oligogenic or polygenic inheritance.

4.1 | Bilineal inheritance of autosomal-dominant polycystic kidney disease

The inheritance of 2 mutations, one each in the *PKD1* and *PKD2* genes, results in more severe phenotype. Either mutation would cause classical ADPKD; the DI does not permit evoking non-Mendelism. The best such case of bilineal disease due to *trans*-heterozygous inheritance of mutations in both genes was a family reported by Pei et al.[13] Two independently segregating mutations explained the initial erroneous impression of lack of linkage to either locus. Obviously, the dual inheritance is compatible with life although the disease severity in 2 patients was worse than when inheriting each mutation separately. With regards to genetic counseling and the risk for disease transmission, each offspring of a doubly affected individual, runs a 50% risk of inheriting either 1 of the 2 mutations alone, 25% of inheriting both and 75% of inheriting any combination of mutations. There is still a 25% likelihood of inheriting none of the mutations.

Interestingly, hypomorphic mutations have been described which singly cause milder or later onset ADPKD. However, the co-inheritance of variants in the genes of *PKD1*, *PKD2*, *PKHD1* and *HNF-1β*, exacerbate the phenotype, even leading to unusually earlier age-at-onset, reminiscent to autosomal-recessive PKD.[30] Also, DI was reported for mutations in the *HNF-1α*, accompanied by maturity-onset diabetes of the young-3 (MODY3), where the second mutation in the *HNF-1β* in some family members caused urogenital and polycystic thyroid changes.[31] In these cases, the co-inheritance of more than one variant better explained the atypical phenotype.

4.2 | Alport syndrome and related collagen-IV glomerulopathies

HTS technologies resulted in 2 reports on patients from 15 families with DI of combinations of mutations in the *COL4A3/A4/A5* genes that confounded the phenotype.[11,32] The Alport phenotype and especially the age-at-onset of ESRD in their 40s, could be better explained by considering the involvement of 2 mutant loci, which depending on the nature of the very genes mutated (autosomal or X-linked) results in complex modes of inheritance with serious implications regarding risk estimation and consequent prognosis. In particular, the fact that *COL4A3* and *COL4A4* are mapped head-to-head on chromosome 2q36-37, makes DI for mutations on alleles in *cis*, to mimic autosomal-dominant inheritance with higher risk, 50% for offspring.

4.3 | Simple calculations of likelihood for coincidental bilineal inheritance

In the absence of solid data regarding the prevalence of true DI, let us embark on calculations for phenotypes that involve diseases of more known frequency, in order to create a feeling on the expected co-inheritance of 2 mutations. How frequently would one expect the co-inheritance of mutations in unlinked genes each one of which causes a monogenic phenotype? Simple calculations are as follows:

Inheritance of dominant mutations in 2 genes, when their population prevalence is 1/500 (familial hypercholesterolemia; autosomal-dominant PKD).

The prevalence of couples with 2 affected spouses: 1/500 × 1/500.

The likelihood for a child inheriting both conditions: 1/500 × 1/500 × 1/4 = 1/1 000 000.

In the UK, one would expect 6 to 7 affected newborns per year. In Greece, one would expect 2 to 3 affected newborns every 3 years. In my country Cyprus, once every about 100 years!

For a frequency of 1/100: 1/100 × 1/100 × 1/4 = 1/40 000.

The co-inheritance of recessive alleles will essentially match that for the respective recessive diseases.

Not many dominant disorders have this high frequency. It is reported that TBMN has an estimated population prevalence of 0.3% to 1%.[33] TBMN is a genetically heterogeneous condition but the most common form is caused by heterozygous mutations in the *COL4A3* or *COL4A4* genes in about 40% to 50% of the cases. So, one expects patients with double heterozygosity to be even rarer than 1/40 000 live births. This is a simplistic approach for the sake of deriving a sense of probability to occur, because in reality the situation is more complex as these 2 genes are linked, mapped next to each other on chromosome 2q36-37, and therefore, one expects co-inheritance of 2 mutations occurring either in *cis* or in *trans* (see example further below). Even much rarer is the situation where one mutation is in the *COL4A3* or *COL4A4* gene and a second is on the X-linked *COL4A5* gene, defects in which are responsible for the most common form of classical AS. With an estimated male population prevalence of 1/5000 (most probably even rarer), the co-inheritance of 2 mutations by a newborn female comes to: 1/3 125 000 (males will not inherit the X-linked *COL4A5* from the affected father).

Probability of father to have 1 X-linked *COL4A5* mutation (X-linked AS): 1/5000.

Probability of mother to have TBMN due to a *COL4A3* or *COL4A4* mutation: 1/313 (simplistically based on an average TBMN prevalence of 0.65%, where *COL4A3/A4* mutations occur in 50% of TBMN patients).

If the *COL4A5* mutation is to be carried by the mother and the *COL4A3/A4* mutation by the father, the probability for either male or female newborns is the same as above (for estimated frequencies of relevant genes, see References [33–35]).

Several examples of coincidental co-inheritance of different diseases have been reported. The great rarity of such occurrences is exemplified by the publication of 1 single report where they describe a young patient who co-inherited ADPKD-type-1 and ARAS. ADPKD is the most common inherited kidney disorder accounting for the fourth most common cause of ESRD (prevalence of 1/500-1000). ARAS affects less than 1/5000 subjects. The patient inherited 3 mutations, 1 in the *PKD1* gene and a homozygous mutation from his Turkish consanguineous parents who were first cousins.[36] Four additional papers reported on co-inheritance of ADPKD with other connective tissue disorders.[5,37–39]

A recently reported case of coincidental inheritance of recessive phenotypes pertains to the Perrault syndrome (PS), characterized by severe hearing loss and primary ovarian insufficiency (POI). In a Pakistani consanguineous family, 6 patients inherited a known mutation in the *CLDN14* gene [p.(Val85Asp)] and developed bilateral sensorineural hearing loss. The proband with PS had co-inherited a homozygous frameshift *SGO2* gene mutation, p.Glu485Lysfs*5, that was considered responsible for the POI. *SGO2* encodes shugoshin 2 and no mutations had been implicated in human disorders before. In mouse, *Sgol2a* (encoding shugoshin-like 2a) is necessary during meiosis in both sexes to maintain the integrity of the cohesin complex that tethers sister chromatids. In support of the pathogenic role of the *SGO2* defect, mutations in other cohesion complex genes also cause infertility.[40] Considering that the proband manifests 2 genetically distinct disorders, deafness and POI, it does not represent true DI, even though they are the hallmark of the PS.

Based on probabilistic calculations and on documented reported cases, the occurrence of DI is expected seldom in clinical diagnostic laboratories; however, if one considers the global population it ought to happen and should be prepared to recognize it, as it is of scientific interest and clinical concern. Nevertheless, one expects deviations from above probabilistic calculations due to varying gene frequencies, genetic drift and founder phenomena, or genetic isolates in studied populations. At the same time many patients might remain undiagnosed, who have inherited unknown genic combinations resulting in phenotypes not considered of familial nature, solely due to our ignorance.

5 | TRUE NON-MENDELIAN DIGENIC INHERITANCE

5.1 | The DIDA database

The DIDA database is a recent development in genetics databases, accumulating information on diseases with DI, along with details on the genes involved, the DNA variants and digenic combinations detected. It is also a good source for statistics (http://dida.ibsquare.be/). DIDA has used the definition of true DI when 2 genic mutations are necessary and sufficient to cause a phenotype and named "Composite" the diseases where there is either independent segregation of phenotypes or the effect of a driver mutation is modified to a variable extend by secondary variations in gene modifiers. According to DIDA, among 258 entries 34.88% are true DI, 29.07% are composite and the rest 36.05% are still not clear. The true DI represent 90 digenic combinations in 54 diseases, with the Bardet-Biedl syndrome being the most represented.[41] With time, HTS technologies will uncover novel DI cases but I feel it is still unpredictable how prevalent they will prove to be. Statistically, it may not be significant at population level but will have tremendous impact on individual patient diagnosis and treatment, as applied to personalized medicine.

5.2 | Selected recent publications on true digenic inheritance

It is not the purpose of this thesis to describe all known diseases that show true unequivocal DI, as this has been attempted by other excellent reviews. A few elegant examples are worth mentioning and discussing them against diseases that are discovered to present with DI but still following Mendelism, except with exacerbation of symptoms.

Perhaps the most easily replicated DI diseases are those caused by mutations in recessive alleles, which when mutated on their own also lead to monogenic autosomal-recessive phenotypes. When these disorders are genetically heterogeneous, one can envision that multiple non-allelic pair-wise combinations of 2 mutations can cause disease. Prime examples are Bardet-Biedl syndrome, Usher syndrome, Deafness, retinitis pigmentosa, and others.[42] However, in some occasions DI was challenged on the basis that it could be explained by other mutational events that included only 1 of the 2 genes.[43,44] This

and other similar examples should alert the health professionals because it influences the mode of action in genetic testing and counseling of involved parties. A comprehensive list of conditions reported until 2013 is included in Table 1 of Reference [6]. However, only a minority of those have been replicated independently (see also Reference [45]).

A particularly interesting case of DI is FSHD, a form of muscular dystrophy with facial and extremity muscle weakness that may progress to involve both upper and lower extremities. On chromosome 4q35.2 telomere there is *DUX4* gene, embedded within a normally hypermethylated region of D4Z4 repeat units, their number ranging from 2 to >100 repeats. The methylation status is determined by a second gene, *SMCHD1*, on chromosome 18p11.32 (encoding structural maintenance of chromosomes flexible hinge domain containing-1). On occasions that the number of D4Z4 repeats are genetically less than 10, the chromatin is hypomethylated and relaxed, and the *DUX4* gene is expressed in skeletal muscle cells, leading to overexpression of stem cells and germline genes, resulting in apoptotic cell death. Another prerequisite is that the *DUX4* gene is found on a permissive haplotype that stabilizes its mRNA through the expression of a proper polyadenylation signal. This leads to a monogenic autosomal-dominant form of FSHD1. On occasions that the *D4Z4* number of repeats is normal and expected to be epigenetically repressed, mutations in the *SMCHD1* gene, result in hypomethylation and *DUX4* gene expression, again only when the *DUX4* gene is embedded in the array of repeats that allow its polyadenylation and stabilization. This leads to FSHD2. In a nutshell, expression of FSHD2 requires a genetic background that allows stable transcripts of *DUX4* and mutations in the *SMCHD1* which will result in epigenetic hypomethylation and transcription of *DUX4*. The molecular phenotype that segregates with the disease is hypomethylation at the D4Z4 locus, on a *DUX4* permissive haplotype.[46,47]

In the field of dilated cardiomyopathy, a new digenic combination with mutations in the Troponin T Type-2 gene (*TNNT2*) and in the Myosin Heavy Polypeptide-7 gene (*MYH7*) was reported in a consanguineous Iranian family. Affected members carried both mutations whereas the carriers of either mutation were asymptomatic. The authors used Whole Exome Sequencing (WES) followed by targeted filtered analysis of the 60 or so genes, normally mutated in this highly heterogeneous condition.[48]

A report of DI serving as an exemplar of the vulnerability of complex protein structures, is CANDLE/PRAAS, a form of proteasome-associated autoinflammatory syndrome.[49] The genes involved are *PSMA3*, *PSMB4*, *PSMB8* and *PSMB9*, in various combinations of loss-of-function double heterozygous mutations. The single gene mutation heterozygous parents of the patients were healthy. The dose of wild-type proteasome complexes might be the key feature in these macromolecular structures, as only 6.25% of the final protein structures are expected to be with no mutant subunits, in double heterozygosity. This excellent work, supported by functional experimentation and modeling, exemplifies the usefulness of WES approaches and highlights that searching for mutations in partners of proteins that participate in large sensitive structures may unravel more cases of DI that may either be misinterpreted as autosomal-recessive inheritance or as incomplete penetrance, when only 1 mutation is inherited.

Neocleous et al published on 3 Cypriot patients with clinically diagnosed familial Mediterranean fever (FMF), the most common hereditary autoinflammatory disease. For yet unclear reasons, it is not unusual for a variable percentage of patients to be heterozygous for mutations in the *MEFV* gene, thus raising the probability of co-inheriting other contributory mutations under DI.[50,51] The authors screened 128 *MEFV* heterozygous patients with FMF-like disease. In addition to the previously found *MEFV* mutation, 2 patients co-segregated heterozygous mutations in the *NLRP3* gene and another patient in the *TNFRSF1A* gene. Both genes are mutated in rare hereditary recurrent fever conditions with dominant inheritance; hence it is worth noting that the heterozygous parents were healthy.[52]

Several reports on renal genetic studies using NGS have identified rare patients with 2 mutations in separate genes but it is not clear whether they act as modifiers to each other or they represent true DI of nephrotic syndrome.[53] In our setup we studied a family where 4 patients have inherited a most likely pathogenic mutation in *COL4A5*, p.Asp654Tyr and a putative contributory mutation in the *LAMA5* gene, p.Pro1243Leu. At least 2 previous reports reported on *LAMA5* variants that were associated with FSGS, thus supporting DI in our patients. In fact, the spectrum of symptoms in 2 males of 57 and 60 years, included hematuria, proteinuria, FSGS, loss of kidney function and renal cortical cysts. Mice with a double *Lama5* knockout are fatal; however, mice with a hypomorphic *Lama5* mutation (Lama5neo) that reduces laminin-α5 expression, exhibit proteinuria, hematuria and cystic kidneys.[54] It is probable, therefore, that although this might not represent true DI, the *LAMA5*-p.Pro1243Leu variant behaves as a hypomorphic mutation that adds up to the Alport background[55]

Evidence for true DI was published while this review was in press, in a Libyan patient with distal renal tubular acidosis (dRTA). Two heterozygous mutations were identified in the genes *ATP6V1B1* and *ATP6V0A4*, which normally are mutated in autosomal recessive forms of dRTA.[56]

Finally, in kidney cyst formation in ADPKD types 1 and 2, the two-hit hypothesis has been documented in numerous cases.[57,58] Specifically, cyst formation was shown to initiate when inheriting a germinal mutation and after the occurrence of a second post-zygotic somatic event that inactivates the other allele of the same gene, *PKD1* or *PKD2*, which had been inherited from the healthy parent. This understanding makes ADPKD a recessive condition at the cellular level as 2 loss-of-function mutations were necessary for clonal cystogenesis.[59,60] Additionally, we and others showed that true DI was sufficient to cause cystogenesis. Careful examination of the DNA of cyst-lining epithelial cells, demonstrated *trans*-heterozygous mutations, where a germinal mutation had been inherited in the *PKD1* (or *PKD2*) gene and a second somatic mutation occurred in 1 allele of the *PKD2* (or *PKD1*) gene.[61,62]

5.3 | Interpretation of pathogenicity of DNA variants

Although HTS technologies are empowering our approaches, they are not particularly assisting us in evaluating the pathogenicity of detected variants. In all truth, they make things more complicated as we generate many more candidate variants with likely pathogenic or modifying role that need to be elucidated. One is tempted to

attribute larger/small driver/modifying roles to novel variants, especially to non-synonymous SNPs or small indels, a task that even with today's technologies is daunting due to limitations on number of patients and the lack of robust functional assays.

The human genome is extremely polymorphic, while every human being carries a number of functionally significant mutations, perhaps in the order of 50 to 100, previously implicated in inherited disorders,[63] which when being true recessive do not confer any recognizable symptoms. There are serious efforts by several consortia, depositing information on validated DNA variants that are not accompanied by perceptible symptoms. Many times the classification of a variant as clearly non-pathogenic is particularly difficult as there are no robust functional assays that would allow an unequivocal permanent settlement, especially in the absence of meticulous deep phenotyping. With regards to approaches taking into consideration the variant population frequency, let us not forget that several recessive mutations have relatively high frequencies and global distribution, causing diseases that include beta-thalassaemia, hemochromatosis, CF, FMF and others. At the same time, it is reasonable to hypothesize that there must be many rare "orphan" mutations that have not been linked yet to known phenotypes/disorders. Many "orphan" mutations are going to be accounted for when studied by HTS, accompanied by deep phenotyping. In fact, some very rare genetic disorders may have not been recognized as heritable yet, only reported as sporadic incidents of unknown heritability.

It has been the experience for many, during the previous years, when screening for mutations with older laborious methods, to terminate the search when we thought we found 1 mutation (or 2, for recessive disorders). Therefore, it is inevitable that in numerous occasions a probable co-inherited additional mutation, either in *cis* or in *trans* was missed. If this is true for monogenic disorders imagine the loss of information we experienced for conditions with digenic or oligogenic inheritance, where other genes of remote function and chromosomal location are involved.

6 | CONCLUDING REMARKS

The complexity of the human genome and its formerly unpredictable polymorphic nature, inevitably leads to coincidental inheritance of 2 or more variants of functional significance. Many unrelated phenotypes might present together simply as the result of this probabilistic occurrence. Importantly, phenotypes primarily caused by a single driver mutation that largely determines the diagnosis, but exacerbated or ameliorated by epistatic effects are expected to be highly prevalent. Such epistatic effects might represent GM the invocation of which better explains the phenotype, as a case of pseudo-DI. One should be prepared to envision not only simple cases where 1 or 2 GM exert a major effect and can explain the pleiotropic phenotype, reminiscent to a phenotypic chameleon. Perhaps a most likely scenario is to expect multiple variants (tens or hundreds) to exert their cumulative effects, in ways that are utterly difficult to identify statistically due to weak association when studied singly. Additionally, the rarity of most monogenic disorders makes it difficult to decipher through the human genome complexity, unless there are a few modifiers with an unusually strong effect or when following the candidate variant approach.

Many not-easily recognizable heritable conditions may exist, due to true DI, masked as non-genetic or sporadic. NGS technologies enable us to bet that many disorders may be the result of digenism

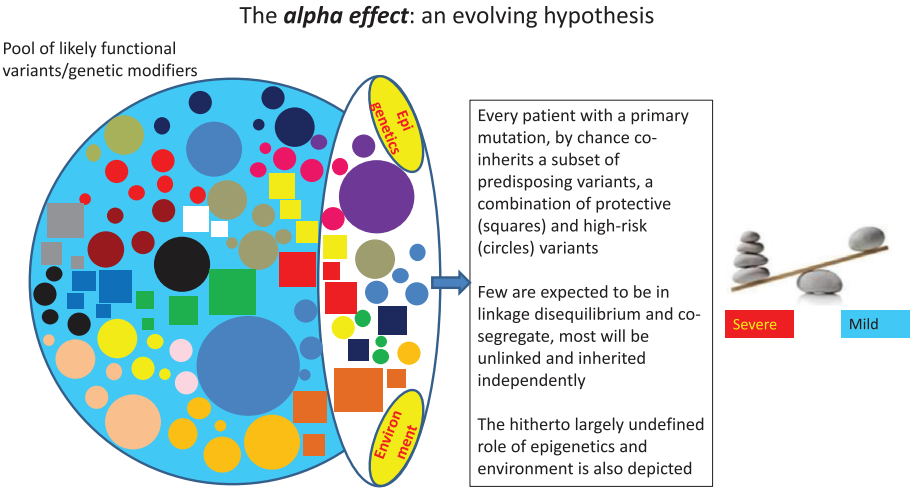

FIGURE 3 Depiction of our evolving hypothesis regarding the complex nature of secondary events that contribute to the broad phenotypic spectrum (phenotypic chameleon). This is what we call the *genetic alpha effect*. Different patients with a defined monogenic disease diagnosis, may inherit additional DNA variants with variable effect size, amongst many in a large functional variant pool. Variants with very large effects (larger shapes) may be adequate to impact phenotype on their own. Others with smaller effect (smaller shapes) may require co-inheritance of several to become noticeable. Shapes with same color are envisioned to represent variants in proteins of common macromolecular complexes or same/converging pathways. It is also predicted that amongst all DNA variants with contributory role, some may increase (circles) while others may decrease (squares) risk to progressive clinical course. Depending on the overall summation and potential synergism between protective and risk factors, the balance may shift and the patient may end-up with a mild or severe disease outcome on long follow-up

involving genes implicated in highly heterogeneous conditions. The combination of HTS technologies and elaborate bioinformatics tools is destined to identify hidden disease entities that are caused by DI or even more complex inheritance. We raise the possibility that in those occasions of genetic heterogeneity that mutations in several genes cause the same recessive disorder, a broad repertoire of double heterozygosities might lead to similar or synthetic phenotypes, not easily elucidated with older technologies. For example, a recessive disorder involving 5 genes predicts for 10 pair-wise combinations of DI and a 6-gene system predicts for 15 pair-wise DI combinations, thus suggesting that true DI might be more frequent than indicated by current data. It does not escape our attention that not all pair-wise mutant combinations will manifest a phenotype.

Finally, we wish to share our proposition for the *genetic alpha effect* hypothesis to describe the contributory role of secondary functional DNA variants, either singly or in concert with others, to configure the final phenotype (Figure 3). The basic concept is that on the background of an inherited monogenic condition, the co-inheritance of one or more such variants from a large pool, which variably increase or decrease the risk to progression, will determine whether the patient will end-up with a more severe or milder end-phenotype. The *alpha effect* provides for tens or hundreds of variants with small or medium effect as well as for fewer or even one, with a larger decisive effect. One fundamental prerequisite of this hypothesis is that in the human genome there are many functional variants, unable to cause a recognizable phenotype on their own, ranging from very rare to very frequent. Most of them remain obscure or masked and waiting to be discovered. We admit this is a simplistic schematic representation of the hypothesis and we hope to refine it as we accumulate more data based on solid experimental evidence.

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Conflict of interest

Nothing to declare.

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REFERENCES

1. Wallis Y, Payne S, McAnulty C et al. Practice Guidelines for the Evaluation of Pathogenicity and the Reporting of Sequence Variants in Clinical Molecular Genetics. Guidelines updated by the Association for Clinical Genetic Science (formally Clinical Molecular Genetics Society and Association of Clinical Cytogenetics) and the Dutch Society of Clinical Genetic Laboratory Specialists approved September 2013), 2013, 16. http://www.acgs.uk.com/media/774853/evaluation_and_reporting_of_sequence_variants_bpgs_june_2013_-_finalpdf.pdf.
2. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17:405–424.
3. Lowik M, Levtchenko E, Westra D, et al. Bigenic heterozygosity and the development of steroid-resistant focal segmental glomerulosclerosis. *Nephrol Dial Transplant*. 2008;23:3146–3151.
4. Drumm ML, Konstan MW, Schluchter MD, et al. Genetic modifiers of lung disease in cystic fibrosis. *N Engl J Med*. 2005;353:1443–1453.
5. Hateboer N, Buchalter M, Davies SJ, et al. Co-occurrence of autosomal dominant polycystic kidney disease and Marfan syndrome in a kindred. *Am J Kidney Dis*. 2000;35:753–760.
6. Schaffer AA. Digenic inheritance in medical genetics. *J Med Genet*. 2013;50:641–652.
7. Sosnay PR, Raraigh KS, Gibson RL. Molecular genetics of cystic fibrosis transmembrane conductance regulator. *Genotype Phenotype Pediatr Clin North Am*. 2016;63:585–598.
8. Jais JP, Knebelmann B, Giatras I, et al. X-linked Alport syndrome: natural history in 195 families and genotype-phenotype correlations in males. *J Am Soc Nephrol*. 2000;11:649–657.
9. Hwang YH, Conklin J, Chan W, et al. Refining genotype-phenotype correlation in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 2016;27:1861–1868.
10. Gropman AL, Adams DR. Atypical patterns of inheritance. *Semin Pediatr Neurol*. 2007;14:34–45.
11. Mencarelli MA, Heidet L, Storey H, et al. Evidence of digenic inheritance in Alport syndrome. *J Med Genet*. 2015;52:163–174.
12. Font-Llitjos M, Jimenez-Vidal M, Bisceglia L, et al. New insights into cystinuria: 40 new mutations, genotype-phenotype correlation, and digenic inheritance causing partial phenotype. *J Med Genet*. 2005;42:58–68.
13. Pei Y, Paterson AD, Wang KR, et al. Bilineal disease and trans-heterozygotes in autosomal dominant polycystic kidney disease. *Am J Hum Genet*. 2001;68:355–363.
14. Sabater-Molina M, Perez-Sanchez I, Hernandez Del Rincon JP, et al. Genetics of hypertrophic cardiomyopathy: a review of current state. *Clin Genet*. 2017. Epub ahead of print.
15. Deltas C, Savva I, Voskarides K, et al. Carriers of autosomal recessive Alport syndrome with thin basement membrane nephropathy presenting as focal segmental glomerulosclerosis in later life. *Nephron*. 2015;130:271–280.
16. Papazachariou L, Papagregoriou G, Hadjipanagi D, et al. Frequent COL4 mutations in familial microhematuria accompanied by later-onset Alport nephropathy due to focal segmental glomerulosclerosis. *Clin Genet*. 2017. Epub ahead of print.
17. Fallerini C, Dosa L, Tita R, et al. Unbiased next generation sequencing analysis confirms the existence of autosomal dominant Alport syndrome in a relevant fraction of cases. *Clin Genet*. 2014;86:252–257.
18. Stefanou C, Pieri M, Savva I, et al. Co-inheritance of functional Podocin variants with heterozygous collagen IV mutations predisposes to renal failure. *Nephron*. 2015;130:200–212.
19. Voskarides K, Stefanou C, Pieri M, et al. A functional variant in NEPH3 gene confers high risk of renal failure in primary hematuric glomerulopathies. Evidence for predisposition to microalbuminuria in the general population. *PLoS One*. 2017;12:e0174274.
20. Voskarides K, Demosthenous P, Papazachariou L, et al. Epistatic role of the MYH9/APOL1 region on familial hematuria genes. *PLoS One*. 2013;8:e57925.
21. Papagregoriou G, Erguler K, Dweep H, et al. A miR-1207-5p binding site polymorphism abolishes regulation of HBEGF and is associated with disease severity in CFHR5 nephropathy. *PLoS One*. 2012;7:e31021.
22. Papazachariou L, Demosthenous P, Pieri M, et al. Frequency of COL4A3/COL4A4 mutations amongst families segregating glomerular microscopic hematuria and evidence for activation of the unfolded protein response. Focal and segmental glomerulosclerosis is a frequent development during ageing. *PLoS One*. 2014;9:e115015.
23. Lennon R, Stuart HM, Bierzynska A, et al. Coinheritance of COL4A5 and MYO1E mutations accentuate the severity of kidney disease. *Pediatr Nephrol*. 2015;30:1459–1465.

24. Mele C, Iatropoulos P, Donadelli R, et al. MYO1E mutations and childhood familial focal segmental glomerulosclerosis. *N Engl J Med*. 2011;365:295–306.
25. Liu M, Shi S, Senthilnathan S, et al. Genetic variation of DKK3 may modify renal disease severity in ADPKD. *J Am Soc Nephrol*. 2010;21:1510–1520.
26. Federico G, Meister M, Mathow D, et al. Tubular Dickkopf-3 promotes the development of renal atrophy and fibrosis. *JCI Insight*. 2016;1:e84916.
27. Louie CM, Caridi G, Lopes VS, et al. AHI1 is required for photoreceptor outer segment development and is a modifier for retinal degeneration in nephronophthisis. *Nat Genet*. 2010;42:175–180.
28. Khanna H, Davis EE, Murga-Zamalloa CA, et al. A common allele in RPGRIP1L is a modifier of retinal degeneration in ciliopathies. *Nat Genet*. 2009;41:739–745.
29. Gallati S. Disease-modifying genes and monogenic disorders: experience in cystic fibrosis. *Appl Clin Genet*. 2014;7:133–146.
30. Bergmann C, von Bothmer J, Ortiz Bruchle N, et al. Mutations in multiple PKD genes may explain early and severe polycystic kidney disease. *J Am Soc Nephrol*. 2011;22:2047–2056.
31. Karges B, Bergmann C, Scholl K, et al. Digenic inheritance of hepatocyte nuclear factor-1alpha and -1beta with maturity-onset diabetes of the young, polycystic thyroid, and urogenital malformations. *Diabetes Care*. 2007;30:1613–1614.
32. Fallerini C, Baldassarri M, Trevisson E, et al. Alport syndrome: impact of digenic inheritance in patients management. *Clin Genet*. 2017;92:34–44.
33. Gregory MC. The clinical features of thin basement membrane nephropathy. *Semin Nephrol*. 2005;25:140–145.
34. Savige J, Rana K, Tonna S, et al. Thin basement membrane nephropathy. *Kidney Int*. 2003;64:1169–1178.
35. Hasstedt SJ, Atkin CL. X-linked inheritance of Alport syndrome: family P revisited. *Am J Hum Genet*. 1983;35:1241–1251.
36. Ebner K, Reintjes N, Feldkotter M, et al. A case report on the exceptional coincidence of two inherited renal disorders: ADPKD and Alport syndrome. *Clin Nephrol*. 2017;88:45–51.
37. Kaplan BS, Kaplan P, Kessler A. Cystic kidneys associated with connective tissue disorders. *Am J Med Genet*. 1997;69:133–137.
38. Somlo S, Rutecki G, Giuffra LA, et al. A kindred exhibiting cosegregation of an overlap connective tissue disorder and the chromosome 16 linked form of autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 1993;4:1371–1378.
39. Hoefele J, Mayer K, Marschall C, et al. Rare co-occurrence of osteogenesis imperfecta type I and autosomal dominant polycystic kidney disease. *World J Pediatr*. 2016;12:501–503.
40. Faridi R, Rehman AU, Morell RJ, et al. Mutations of SGO2 and CLDN14 collectively cause coincidental Perrault syndrome. *Clin Genet*. 2017;91:328–332.
41. Gazzo AM, Daneels D, Cilia E, et al. DIDA: a curated and annotated digenic diseases database. *Nucleic Acids Res*. 2016;44:D900–D907.
42. Beales PL, Badano JL, Ross AJ, et al. Genetic interaction of BBS1 mutations with alleles at other BBS loci can result in non-Mendelian Bardet-Biedl syndrome. *Am J Hum Genet*. 2003;72:1187–1199.
43. Rodriguez-Paris J, Tamayo ML, Gelvez N, et al. Allele-specific impairment of GJB2 expression by GJB6 deletion del(GJB6-D13S1854). *PLoS One*. 2011;6:e21665.
44. Rodriguez-Paris J, Schrijver I. The digenic hypothesis unraveled: the GJB6 del(GJB6-D13S1830) mutation causes allele-specific loss of GJB2 expression in *cis*. *Biochem Biophys Res Commun*. 2009;389:354–359.
45. Cooper DN, Krawczak M, Polychronakos C, et al. Where genotype is not predictive of phenotype: towards an understanding of the molecular basis of reduced penetrance in human inherited disease. *Hum Genet*. 2013;132:1077–1130.
46. Lemmers RJ, Tawil R, Petek LM, et al. Digenic inheritance of an SMCHD1 mutation and an FSHD-permissive D4Z4 allele causes facioscapulohumeral muscular dystrophy type 2. *Nat Genet*. 2012;44:1370–1374.
47. Lupski JR. Digenic inheritance and Mendelian disease. *Nat Genet*. 2012;44:1291–1292.
48. Petropoulou E, Soltani M, Firoozabadi AD, et al. Digenic inheritance of mutations in the cardiac troponin (TNNT2) and cardiac beta myosin heavy chain (MYH7) as the cause of severe dilated cardiomyopathy. *Eur J Med Genet*. 2017;60:485–488.
49. Brehm A, Liu Y, Sheikh A, et al. Additive loss-of-function proteasome subunit mutations in CANDLE/PRAAS patients promote type I IFN production. *J Clin Invest*. 2015;125:4196–4211.
50. Rossou E, Kouvatsi A, Aslanidis C, et al. Multiplex molecular diagnosis of MEFV mutations in patients with familial Mediterranean fever by LightCycler real-time PCR. *Clin Chem*. 2005;51:1725–1727.
51. Deltas CC, Mean R, Rossou E, et al. Familial Mediterranean fever (FMF) mutations occur frequently in the Greek-Cypriot population of Cyprus. *Genet Test*. 2002;6:15–21.
52. Neocleous V, Byrou S, Toumba M, et al. Evidence of digenic inheritance in autoinflammation-associated genes. *J Genet*. 2016;95:761–766.
53. Chatterjee R, Hoffman M, Cliften P, et al. Targeted exome sequencing integrated with clinicopathological information reveals novel and rare mutations in atypical, suspected and unknown cases of Alport syndrome or proteinuria. *PLoS One*. 2013;8:e76360.
54. Shannon MB, Patton BL, Harvey SJ, et al. A hypomorphic mutation in the mouse laminin alpha5 gene causes polycystic kidney disease. *J Am Soc Nephrol*. 2006;17:1913–1922.
55. Voskarides K, Papagregoriou G, Hadjipanagi D, et al. COL4A5 and LAMA5 variants co-inherited in familial hematuria: digenic inheritance or genetic modifier effect? *BMC Nephrology*. In Press.
56. Nagara M, Papagregoriou G, Ben Abdallah R, et al. Distal renal tubular acidosis in a Libyan patient: Evidence for digenic inheritance. *Eur J Med Genet*. 2017. https://doi.org/10.1016/j.ejmg.2017.10.002. Epub ahead of print.
57. Qian F, Watnick TJ, Onuchic LF, et al. The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type I. *Cell*. 1996;87:979–987.
58. Wu G, D'Agati V, Cai Y, et al. Somatic inactivation of Pkd2 results in polycystic kidney disease. *Cell*. 1998;93:177–188.
59. Pei Y, Watnick T, He N, et al. Somatic PKD2 mutations in individual kidney and liver cysts support a "two-hit" model of cystogenesis in type 2 autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 1999;10:1524–1529.
60. Koptides M, Hadjimichael C, Koupepidou P, et al. Germinal and somatic mutations in the PKD2 gene of renal cysts in autosomal dominant polycystic kidney disease. *Hum Mol Genet*. 1999;8:509–513.
61. Koptides M, Mean R, Demetriou K, et al. Genetic evidence for a trans-heterozygous model for cystogenesis in autosomal dominant polycystic kidney disease. *Hum Mol Genet*. 2000;9:447–452.
62. Watnick T, He N, Wang K, et al. Mutations of PKD1 in ADPKD2 cysts suggest a pathogenic effect of trans-heterozygous mutations. *Nat Genet*. 2000;25:143–144.
63. Genomes Project Consortium, Abecasis GR, Altshuler D, et al. A map of human genome variation from population-scale sequencing. *Nature*. 2010;467:1061–1073.

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