Novel pedigree analysis implicates DNA repair and chromatin remodeling in Multiple Myeloma risk

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ABSTRACT

The high-risk pedigree (HRP) design is an established strategy to discover rare, highly-penetrant, Mendelian-like causal variants. Its success, however, in complex traits has been modest, largely due to challenges of genetic heterogeneity and complex inheritance models. We describe a HRP strategy that addresses intra-familial heterogeneity, and identifies inherited segments important for mapping regulatory risk. We illustrate this new Shared Genomic Segment (SGS) method in 11 extended, Utah, multiple myeloma (MM) HRPs, and subsequent exome sequencing in SGS regions of interest in 1064 MM/MGUS (monoclonal gammopathy of undetermined significance – a precursor to MM) cases and 964 controls from a jointly-called collaborative resource, including cases from the initial 11 HRPs. One genome-wide significant 1.8 Mb shared segment was found at 6q16. Exome sequencing in this region revealed predicted deleterious variants in *USP45* (p.Gln691*, p.Gln621Glu), a gene known to influence DNA repair through endonuclease regulation. Additionally, a 1.2 Mb segment at 1p36.11 is inherited in two Utah HRPs, with coding variants identified in *ARID1A* (p.Ser90Gly, p.Met890Val), a key gene in the SWI/SNF chromatin remodeling complex. Our results provide compelling statistical and genetic evidence for segregating risk variants for MM. In addition, we demonstrate a novel strategy to use large HRPs for risk-variant discovery more generally in complex traits.

AUTHOR SUMMARY

Although family-based studies demonstrate inherited variants play a role in many common and complex diseases, finding the genes responsible remains a challenge. High-risk pedigrees, or families with more disease than expected by chance, have been helpful in the discovery of variants responsible for less complex diseases, but have not reached their potential in complex diseases. Here, we describe a method to utilize high-risk pedigrees to discover risk-

genes in complex diseases. Our method is appropriate for complex diseases because it allows for genetic-heterogeneity, or multiple causes of disease, within a pedigree. This method allows us to identify shared segments that likely harbor disease-causing variants in a family. We demonstrate our method in Multiple Myeloma, a heritable and complex cancer of plasma cells. We identified two genes *USP45* and *ARID1A* that fall within shared segments with compelling statistical evidence. Exome sequencing of these genes revealed likely-damaging variants inherited in Myeloma high-risk families, suggesting these genes likely play a role in development of Myeloma. Our Myeloma findings demonstrate our high-risk pedigree method can identify genetic regions of interest in large high-risk pedigrees that are also relevant to smaller nuclear families and overall disease risk. In sum, we offer a strategy, applicable across phenotypes, to revitalize high-risk pedigrees in the discovery of the genetic basis of common and complex disease.

INTRODUCTION

Rare risk variants have been suggested as a source of missing heritability in the majority of complex traits [1–3]. High-risk pedigrees (HRPs) are a mainstay for identifying rare, highly penetrant, Mendelian-like causal variants [4–11]. However, while successful for relatively simple traits, genetic heterogeneity remains a major obstacle that reduces the effectiveness of HRPs for gene mapping in complex traits [12,13]. Also challenging is mapping regulatory variants, likely to be important for complex traits, necessitating interrogation outside the well-annotated coding regions of the genome [14,15]. Localizing chromosomal regions to target the search for rare risk variants will be instrumental in mapping them.

Here we develop a HRP strategy based on our previous Shared Genomic Segment (SGS) approach [16] that focuses on pedigrees sufficiently large to singularly identify

segregating chromosomal segments of statistical merit. The method addresses genetic heterogeneity by optimizing over all possible subsets of studied cases in a HRP. Key to the utility of the method is the derivation of significance thresholds for interpretation. These thresholds address the genome-wide search and the multiple testing, inherent from the optimization, through use of distribution fitting and the Theory of Large Deviations.

We apply this novel method to 11 MM HRPs, and use exome sequencing from a collaborative resource of 68 multiplex MM pedigrees to perform subsequent targeted searches at the variant level. MM is a complex cancer of the plasma cells with 30,330 new cases annually (incidence 6.5/100,000 per year) [17]. Despite survival dramatically increasing from 25.8% in 1980 to 48.5% in 2012, MM remains a cancer with one of the lowest 5-year survival rates in adult hematological malignancies [17]. MM is preceded by a condition referred to as monoclonal gammopathy of undetermined significance (MGUS). Evidence for the familial clustering of MM is consistently replicated [18–21], as is its clustering with MGUS [22–25]. Genetic pedigree studies in MM are scarce as it remains a challenge to acquire samples in pedigrees due to rarity and low survival rates. The Utah MM HRPs are one of only a few pedigree resources worldwide and contains unparalleled multi-generational high-risk pedigrees. Thus far, no segregating risk variants have been identified for MM.

RESULTS

Pedigree analysis strategy

We developed a gene mapping strategy, based on the SGS method [16,26], that accounts for intra-familial heterogeneity and multiple testing. The basic SGS method identifies genomic segments shared identical-by-state (sharing without regard to inheritance) between a defined set of cases using a dense genome-wide map of common single nucleotide

polymorphisms (SNPs), either from a genotyping platform or extracted from sequence data. If the length of a shared segment is significantly longer than by chance, inherited sharing is implied; theoretically, chance inherited sharing in distant relatives is extremely improbable. Nominal chance occurrence (nominal p-value) for shared segments is assessed empirically using gene-drop simulations to create a null distribution, as follows. Null genotype configurations are generated by assigning haplotypes to pedigree founders according to a publicly available linkage disequilibrium (LD) map, followed by segregation of these through the pedigree structure to the case set via simulated Mendelian inheritance according to a genetic (recombination) map. Gene-drops are performed independent of disease status and the resulting genotype data in the case set are representative of chance sharing. This basic method was shown to have excellent power in homogeneous pedigrees [16].

In our new strategy, we iterate over all non-trivial combinations of the cases (subsets) in each pedigree to address heterogeneity in a “brute-force” fashion. For each subset, shared segments throughout the genome are identified and nominal p-values assigned. Across subsets, an optimization procedure is performed at every marker across the genome to identify the segment with the most significant sharing evidence. All shared segments selected by the optimization procedure, and their respective p-values, comprise the final optimized SGS results.

To perform significance testing and identify segments that are unexpected by chance (hypothesized to harbor risk loci), we derive significance thresholds to account for the genome-wide optimization. Acknowledging that the vast majority of observed sharing across a genome is under the null (true risk loci are a very small minority of the genome), we use the observed optimized results ($Y = -log_{10}(p)$) to model the distribution for optimized SGS results. We note that this approach may be slightly conservative because signals for true risk loci are also included. We identified the gamma distribution as adequate to represent the distribution (Fig. 1). Based on the fitted distribution, $Y \sim \Gamma(k, \sigma)$, where k and σ and the shape and scale parameters,

we apply the Theory of Large Deviations; previously applied to successfully model genome-wide fluctuations in linkage analysis [27]. The significance threshold, T, accounts for multiple testing of optimized segments across the genome, and is found by solving Eq. 1:

$$\mu(X) = [C + 2GX]\alpha(X) \qquad (1)$$

where $T = 10^{-X\sigma/2}$, $X = 2Y/\sigma \sim \chi^2_{2k}$, $\mu(X)$ is the genome-wide false positive rate required, $\alpha(X)$ is nominal probability of exceeding X, C is the number of chromosomes considered, and G is the genome length in Morgans. A criterion of $\mu(X) = 0.05$ is typically used to define the genome-wide significant threshold (false positive rate of 0.05 per genome), and $\mu(X) = 1$ to define the genome-wide suggestive threshold (false positive rate of 1 per genome).

In general, we found that the fitted distributions were sufficiently stable to produce robust significance thresholds after 100,000-300,000 simulations (Table 1). Typically, threshold determination requires 1,000-3,000 CPU hours per pedigree, increasing with the number of subsets and separating meioses between pedigree cases. For example, in pedigree UT-571744, 300k simulations genome-wide (2,513,408 segments) took 1,275 CPU hours on tangent nodes featuring Intel Xeon E5-2650 processors. Once significance thresholds are established, subset/segment combinations of potential interest are identified and additional simulations are restricted to those combinations to gain the required p-value resolution. For these subsequent targeted simulations, we use a marginalized LD map specific for the segment of interest, dramatically reducing the analysis time. For example, in pedigree UT-571744, 600M simulations on one segment took 325 CPU hours on tangent nodes featuring Intel Xeon E5-2650 processors. See S1 Fig. for an overview of the strategy pipeline.

Table 1. Genome-wide Significance Thresholds. Fitted distributions are stable enough for threshold determination after 100,000 to 300,000 simulations.

Pedigree	100k	200k	300k	1M
260	6.36×10^{-6}	6.35×10^{-6}	6.28×10^{-6}	6.25×10^{-6}
576834	3.50×10^{-6}	3.53×10^{-6}	3.53×10^{-6}	3.51×10^{-6}
571744	3.80×10^{-6}	3.83×10^{-6}	3.75×10^{-6}	3.80×10^{-6}
34955	5.67×10^{-6}	5.60×10^{-6}	5.61×10^{-6}	5.61×10^{-6}

Application to Utah, MM HRPs

We applied our new pedigree analysis strategy to 11 Utah MM HRPs using high-density OMNI Express SNP array genotype data. Each pedigree was selected to contain excess MM (4-37 MM total per pedigree), had 2-4 sampled MM cases with genotype data, and 8-23 meioses per pedigree between the sampled cases. After quality control, a consistent set of 678,447 SNPs were used for all SGS analyses. The total number of shared segments for each pedigree across all subsets ranged from 638,525 to 6,765,500 (larger pedigrees with more subsets producing larger numbers of segments). After optimization, $Y = -log_{10}(p)$ for 6,697 to 10,369 segments were fit to gamma distributions for each pedigree, and used to determine genome-wide significant and suggestive thresholds (Eq. 1). The genome-wide significant thresholds ranged from 6.2×10^{-5} to 7.8×10^{-7} and genome-wide suggestive from 8.2×10^{-4} to 2.1×10^{-5} (S1 Table).

A genome-wide significant, 1.8 Mb shared segment (p = 3.3x10-6) was observed in pedigree UT-571744. All three genotyped MM cases, separated by 20 meioses, share the segment (Fig. 2a and Table 2). The segment is located at chromosome 6q16 (98.49-100.24 Mb; hg19) and includes 9 genes: *POU3F2*, *FBXL4*, *FAXC*, *COQ3*, *PNISR*, *USP45*, *TSTD3*, *CCNC*, and *PRDM13* (Figure 2b).

Table 2. Significant or overlapping SGSs and segregating SNVs.

Family	Cases	Me	Position	Len	p-value	Gene	Conseq	Impact	AAF
UT 571744	3	20	6:98,489,655—100,243,996	1.8	$3.3x10^{-6}$*				
PET-Nice 0909	3(2)	3	6:99,891,443			*USP45*	p.Gln691*	SG	None
Mayo 458	2(1)	2	6:99,893,787			*USP45*	p.Gln621Glu	MS	None
UT 576834	3	12	1:24,389,214—33,298,821	8.9	$3.0x10^{-4}$				
UT 260	3	16	1:26,224,634—27,384,988	1.2	$2.1x10^{-4}$				
UT 576834	3	12	1:27,023,162			*ARID1A*	p.Ser090Gly	MS	0.0000
Cornell MM12	2	4	1:27,089,712			*ARID1A*	p.Met890Val	MS	0.0001

Legend: UT – Utah, Cases – total MM and MGUS cases (number of MGUS), Me – meioses, Position – build HG19, Len – length in mega-bases, p-value – SGS p-value, *genome-wide significant, Conseq – exome-variant consequence, SG – stop gain variant, MS – missense variant, AAF – alternate allele frequency based on the non-TCGA, non-Finnish, European ExAC individuals.

We also identified two HRPs, UT-576834 and UT-260, with overlapping shared segments at 1p36.11 (Fig. 3). A 8.9 Mb (24.39-33.30 Mb, p = 3.0×10-4) segment was observed in 3 of the 4 genotyped MM cases in UT-576834, shared across 12 meioses (Fig. 3b and Table 2). A nested 1.2 Mb shared segment (26.22-27.38 Mb; p = 2.1×10-4) segregated to 3 MM cases separated by 16 meioses in UT-260 (Fig. 3a and Table 2). The overlapping segment contains 30 genes (Fig. 3d).

Exome follow-up of shared segments in HRPs

Whole-exome sequencing (WES) data was interrogated, targeted to the shared segments, to identify potential risk variants in the pedigree sharers in the HRP and in a broader set of 57 pedigrees. WES data was available for: 28 cases from the 11 extended Utah HRPs; and 162 exomes from 57 densely clustered MM/MGUS families from Mayo Clinic Rochester,

Weill Cornell, Memorial Sloan Kettering Cancer Center, International Agency for Research on Cancer, and INSERM France (S2 Table). Prioritization was used to identify variants that were: in the target segment; rare (alternate allele frequency, AAF<0.001), potentially deleterious (variant impact predicted to be high or moderate); and observed recurrently in the appropriate segment sharers (if observed in the segment discovery pedigree).

At 6q16, no rare, potentially deleterious coding risk variants were shared by the 3 UT-571744 MM cases in the 1.8 Mb genome-wide significant segment, indicating non-coding regulatory variants may be responsible for MM risk in this pedigree. However, two, rare coding and potentially deleterious single nucleotide variants (SNVs) were identified in two MM/MGUS families (Fig. 2c-e and Table 2). Both SNVs are the in hydrolase domain of *USP45*: a stop gain (p.Gln691*) shared by 3 sibling cases (1 MM and 2 MGUS) in an INSERM family (PET-Nice 0909) and a missense SNV (p.Gln621Glu) shared by 2 siblings (1 MM and 1 MGUS) but not their 2 screened unaffected siblings in Mayo family 485. Coverage of these positions in ExAC sequence data is high (> 99% of the 60,706 ExAC samples had at least 10x read coverage) and neither variant was observed.

Pedigree exomes in the 1.2 Mb segment at 1p36.11 revealed two, rare and potentially deleterious SNVs. The first in discovery pedigree UT-576834: a missense SNV (rs752026201, p.Ser90Gly, AAF = 0.0016 in ExAC) in *ARID1A* (Fig. 3e) shared by 3 of the 4 Utah MM cases, concordant with the segment sharing pattern. A second rare, missense SNV in *ARID1A* (rs140664170, p.Met890Val, AAF < 0.0001 in ExAC) was found to be carried by a pair of MM cousins in Weill-Cornell family 12 (Fig. 3c and e, and Table 2). Based on the ExAC data, *ARID1A* is extremely intolerant to missense variants (Z = 4.1) and loss of function (LoF) SNVs (pLI = 1) [28].

Pathway follow-up of candidate genes

Our SGS findings and pedigree WES identify *USP45* and *ARID1A* as candidate genes for inherited MM risk. We further investigated shared segments and WES for evidence supporting the complexes USP45 and ARID1A are involved in. Here we further expanded our WES to: 154 early-onset MM/MGUS cases from our collaborative group, 733 sporadic MM cases from dbGaP [29], and 964 controls [30].

USP45 is an essential DNA repair regulator, de-ubiquitylating ERCC1 to allow for DNA translocation of the ERCC1-ERCC4 endonuclease [31,32]. This endonuclease is a part of the global genome nucleotide-excision repair (GG-NER) incision complex, a 22 protein complex essential to removing lesions from DNA and cancer prevention [33–36] (see S3 Table). We reviewed SGS results in the Utah HRPs at the location of these 22 genes and identified a genome-wide suggestive segment in pedigree UT-34955 (S2 Fig.). This HRP identified a 0.8 Mb segment at 19q13 (45.71-46.51 Mb; hg19), containing 31 genes including *ERCC1* and *ERCC2* (S2 Fig. and S4 Table). The segment is shared by 3 MM cases separated by 16 meioses ($p = 6.6\times10\text{-}5$). No rare, coding variants were identified from the WES in the 3 MM cases in UT-34955, nor in the remaining 67 pedigrees/families. We interrogated the 22 GG-NER incision complex genes in our 885 early-onset and sporadic MM exomes. This identified a ClinVar-annotated pathogenic, missense SNV in *ERCC4* (p.Arg799Trp) in one early-onset MM case and one sporadic MM case, and a stop-gain SNV in *ERCC3* (p.Arg574Ter), in the same domain as a ClinVar-annotated pathogenic variant, in a second early-onset MM case (S4 Table). Further, burden testing in all MM cases vs controls was significant in 7 of the 22 GG-NER genes: *ERCC3*, *PARP1*, *GTF2H1*, *GTF2H2*, *DDB1*, *RPA2*, and *CHD1L* (S3 Table).

ARID1A is a member of the SWI/SNF chromatin remodeling complex, a 15 gene complex involved in DNA transcription regulation [37] (see S5 Table). Members of this complex

are mutated in >20% of malignancies [38–40], but are extremely intolerant to LoF and missense variation [41] (S5 Table). We reviewed SGS results in the Utah HRPs at the location of these 15 genes and identified a marginal, genome-wide suggestive segment in pedigree UT-549917 shared by 4 MM cases across 21 meioses (p = 2.17×10-5, S3 Fig. and S6 Table). This 1.5 Mb segment at chr3p21.1-p21.2 (52.01-53.56 Mb; hg19) contains 32 genes including *PBRM1* from the SWI/SNF complex. No coding variants were identified in this gene in UT-549917, nor in the remaining 67 pedigrees/families. Burden testing was significant for 8 of the 15 genes in the complex: *ARID1A*, *ARID1B*, *ARID2*, *SMARCA4*, *ACTL6A*, *SMARCD3*, *SMARCC2*, and *SMARCE1* (S5 Table).

DISCUSSION

We developed a novel strategy to identify segregating chromosomal segments shared by subsets of cases in HRPs. It focuses on extended HRPs that are singularly powerful to identify significant genetic segregation. Our strategy allows for genetic heterogeneity within such pedigrees and provides formal significance thresholds for valid interpretation. Previously, extended HRP have not delivered on their potential in complex traits because in common, complex traits, HRPs are likely enriched for multiple susceptibility variants and may capture both familial and sporadic cases in their branches. Our optimization strategy over subsets is attractive because it allows for heterogeneity without prior knowledge of genetic similarities or deep phenotyping. Application of the method to extended MM pedigrees demonstrated the utility of this new method and illustrated that the segments identified were used successfully to narrow the search for risk variants in smaller pedigrees, allowing for an overall strategy that can utilize both large pedigrees and smaller families together for discovery (see Table 2, Fig. 1 and Fig. 2). Post-hoc, additional value can be gained from demographic and/or clinical data on the sharing

subsets shedding light on other shared characteristics that may aid future mapping. Also, we note that in the absence of any significant findings, genome-wide SGS results can be used as genomic annotations of segregation evidence for more heuristic approaches.

While we identified several rare, potentially deleterious coding variants of interest, several of the SGS discovery pedigrees had no coding variants that satisfied prioritization criteria. We believe this will be characteristic of complex traits and that regulatory variants will also play a substantial role. Mutations with strong causal likelihood found in other disease cohorts may focus the search for regulatory variation to particular genes within a shared segment, as with *USP45* in MM. In the absence of such compelling evidence, a return to pedigree segregation methods will provide identification of statistically compelling regions which can concentrate efforts to identify and characterize regulatory risk variants. Our proposed method is a new analytic tool with the potential to reinvigorate the use of extended HRPs in the identification of risk variants that contribute to common, complex disease.

Multiple myeloma is a malignancy of the plasma cells that has been shown to be familial [42]. Consistent with a role for genetics, case-control studies have been successful in identifying association signals for 17 low-risk variants [43–47]. However, despite consistent evidence for familial clustering, our study is the first to explore high-risk MM pedigrees. Using the unique genealogical database available in Utah, we identified and studied extended MM HRPs. We identified a genome-wide significant segment containing *USP45*, an important regulator of DNA repair (see Fig. 1 and Table 2), and a genome-wide suggestive segment harboring other genes in the endonuclease regulation pathway (*ERCC1* and *ERCC2*). Exome sequencing in a collaborative resource of high-risk families and early-onset cases revealed four rare, potentially deleterious coding variants; two novel variants in *USP45* segregating in two pedigrees and two variants in early-onset cases in ERCC3 and ERCC4, the latter annotated as pathogenic in ClinVar. Burden testing including sporadic MM, and comparing to controls, identified significant

enrichment for variants in MM cases in 7 of the 22 GG-NER genes in the protein endonuclease regulation complex.

In particular, the functional literature supports *USP45* as a candidate cancer risk gene. USP45 has been shown to deubiquitylate ERCC1, a catalytic subunit of the ERCC1-ERCC4 DNA repair endonuclease (ERCC4 also known as XPF) [31]. This endonuclease is a critical regulator of DNA repair processes [34]. The complex repairs recombination, double strand break, and inter-strand crosslink by cutting DNA overhangs around a lesion, degrades 3' G-rich overhangs in telomere maintenance, and plays a role in cancer prevention and in tumor resistance to chemotherapy [31,34]. Mouse models have shown USP45 knockout cells have higher levels of ubiquitylated ERCC1 and that cells are hypersensitive to UV radiation and DNA inter-strand cross-links, repair of UV-induced DNA damage, and ERCC1 translocation to DNA damage is impaired [31]. Hence, the deubiquitylase activity of USP45 is important for maintaining the DNA repair ability of ERCC1-ERCC4. In total, these observations implicate the GG-NER pathway and specifically the interaction of USP45 and the disruption of the ERCC1-ERCC4 role in DNA repair as a mechanism of potential importance in MM risk.

Our strategy also identified shared segments overlapping at chr1p36.11 in two Utah pedigrees containing *ARID1A* (Fig. 2) and a genome-wide suggestive segment in a third pedigree harboring another gene in the SWI/SNF complex (*PBRM1*). For the SWI/SNF complex, exome sequencing revealed two rare, potentially deleterious variants in *ARID1A* segregating in two pedigrees. Burden testing provided further evidence for enrichment of variants in *ARID1A* specifically, and in 7 of the 15 genes in the complex. As a component of the SWI/SNF chromatin remodeling complex, ARID1A facilities gene activation by assisting transcription machinery gain access to gene targets [48]. Based on the patterns of mutations in tumor cells, *ARID1A* likely functions as a tumor-suppressor [49]. Members of the SWI/SNF chromatin remodeling complexes are mutated in 20% of malignancies [38], but are extremely

intolerant to LoF and missense variation [41] (see S5 Table). Blockage of chromatin remodeling may sustain cancer development [39]. Aberrant chromatin remodeling contributes to the pathogenesis of ovarian clear-cell carcinoma [49]. It has previously been shown that *ARID1A* is intolerant to variation (LoF and missense mutations) [28], consistent with its prominent somatic role in multiple tumors [38,49,50], including hematological malignancies [51–53]. These observations implicate the SWI/SNF chromatin remodeling complex, and specifically *ARID1A* in MM risk.

This study has limitations. First, the method is applicable only to extended HRPs that are singularly effective for identifying segregating segments (15 meioses between cases is optimal [16]). The method is not directly applicable to the many smaller family-based resources that have been gathered in the complex trait field and may therefore result in findings from single large pedigrees that are private and difficult to replicate. However, as illustrated in our example, in a collaborative setting containing both extended HRPs and smaller families, the approach can be mutually beneficial. Second, our observation of two borderline genome-wide suggestive overlapping segments at 1p36 led to our identification of *ARID1A* as a potential candidate risk gene and illustrates the potential for discoveries using overlapping subthreshold evidence. However, it raises analytical questions of how to systematically identify such segments. This segment would have been ignored based on strict individual-pedigree thresholds and highlights an important area for further methodological development. Finally, this study is observational and cannot describe causation. We have identified two complexes, several genes and specific variants as compelling candidates involved in MM risk, but further functional studies will be required to determine and characterize the mechanisms involved in risk.

In conclusion, we have developed a strategy for gene mapping in complex traits that accounts for heterogeneity within HRPs and formally corrects for multiple testing to allow for statistically rigorous discovery. We applied this strategy to MM, a complex cancer of plasma

cells, and identified multiple shared segments containing genes in nucleotide excision repair and SWI/SNF chromatin remodeling. Exome follow-up supported these segments in both the Utah large HRPs and smaller families from other sites. Our study offers a novel technique for HRP gene mapping and demonstrates its utility to narrow the search for risk-variants in complex traits.

METHODS

SGS Analysis in Utah, Myeloma HRPs

HRPs and genotyping. All participants were studied with informed consent under protocols approved by the University of Utah IRB. Using the statewide Utah Cancer Registry (UCR), all living individuals with MM in Utah were invited to participate and peripheral blood was collected for DNA extraction. Participants were linked in the Utah Population Database (UPDB), a unique resource that integrates UCR records with a 5M person genealogy. HRPs were defined as pedigrees containing statistical excess of MM ($p<0.05$), based on sex and cohort-specific rates in Utah. Eleven of the HRPs identified in the UPDB contained 3-4 MM cases with DNA (total MM cases per pedigree ranged from 4 to 37) with 8-23 meioses between studied MM cases. DNA from the 28 cases was genotyped on the Illumina Omni Express high-density SNP array.

Genotype quality control. Only bi-allelic SNPs were considered. Genotypes and individual call-rates were used to ensure high quality data. PLINK was used to remove SNPs with < 95% call rate across individuals [54]. The final SNP set contained 678,447 single nucleotide variants. After SNP removal for low call rates, individuals were removed based on < 90% call rate across the genome, or if they failed the PLINK sex check. One MM case was removed. The QC'ed SNP data were transformed to match strand orientation of the 1000Genomes.

Probability of sharing a segment. SGS analysis identifies contiguous SNPs that are shared identical-by-state (IBS) by cases in a HRP and assigns an empirical probability of chance ancestral sharing [26]. First, a set of cases in a HRP are defined and all segments of contiguous SNPs shared IBS are identified. Only shared segments > 20 SNPs are considered as lengths shorter than 20 are commonly shared between unrelated individuals. Second, population-based data (here we used CEU and GBR data from the 1000Genomes Project [55]) are used to estimate a graphical model for linkage disequilibrium (LD) [56], providing a probability distribution of chromosome-wide haplotypes in the population. Third, pairs of haplotypes are randomly assigned to pedigree founders according to the haplotype distribution. Founders are individuals whose parents are not specified in the pedigree. For chromosome-wide haplotype simulations the full chromosome LD model is used. Fourth, Mendelian segregation and recombination are simulated to generate genotypes for all pedigree members. The Rutgers genetic map [57] is used for a genetic map for recombination, with interpolation based on physical base pair position for SNPs not represented. Steps two through four create one simulated data set, a random sample from the null hypothesis. This process is repeated hundreds of thousands to millions of times.

Each shared segment in the real data (step one) is compared to the simulated segments at the precise genomic location. The number of times the null segment equals or encompasses the observed segment is counted and divided by the total number of simulations to generate the empirical nominal p-value for the observed shared segment. The simulations continue until a p-value has been estimated to a required resolution, or until it surpasses a defined significance threshold. To facilitate this in an efficient manner, we follow-up specific segments using marginal distributions from the LD model, established using standard graphical modeling methods [58]. The marginalized LD model encompassing only the region of interest, but

capturing relevant LD to accurately simulate genotypes from this region alone. This reduction in markers vastly increases the speed in which simulations are generated. The graphical model estimation, marginalization, and simulation processes are computationally efficient requiring time and storage that is linear with the number of SNPs being considered.

Heterogeneity optimization. We systematically perform SGS analysis on each subset of cases in a HRP. At each marker position across the genome, the optimized segment is the one minimizing the p-value across all subsets considered. All segments selected by the optimization procedure, and their respective p-values, comprise the final optimized SGS results.

Significance threshold determination. A transformation, $Y = -log_{10}(p)$ is performed to the optimized genome-wide SGS p-value vector. The results are fit to a gamma distribution using the MLE method. $Y \sim \Gamma(k, \sigma)$ (k shape, σ scale parameterization). The Theory of Large Deviations has previously been used in pedigree studies to model extreme values in a genome wide genetic setting [27], and it has been shown that for a statistic following a Gaussian distribution, the number of segments where the statistic exceeds a threshold W has mean:

$$\mu(W) = [C + 2\rho G W^2]\alpha(W) \qquad (2),$$

where $\alpha(W)$ is the pointwise significance level of exceeding W, C is the number of chromosomes considered, ρ reflects the recombination rate ($\rho = 1$ for general pedigrees), and G is genetic length in Morgans. Lander & Kruglyak demonstrated that the same equation extends a statistic following the chi-squared distribution:

$$\mu(X) = [C + 2\rho G X]\alpha(X) \qquad (3),$$

based on the distributional relationship between the chi-squared and Normal distributions $W^2 = X$. Here, we use the distributional relationship between the gamma and chi-square distributions,

our estimated k and σ gamma parameters, where $T = 10^{-X\sigma/2}$, $X = 2Y/\sigma \sim \chi^2_{2k}$, and the genetic length of the genome (matched to that used in the gene-drop) to utilize Eq. 3 and derive $\mu(X)$ thresholds. Solving for $\mu(X) = 0.05$ and $\mu(X) = 1$ produced significance and suggestive thresholds, respectively. These thresholds are remarkably stable after a few hundred thousand simulations. For pedigrees with very large numbers of meioses (>50) between the full case-set a larger number of simulations may be required.

Interrogating target regions defined by shared segments in WES

Participants. WES data were interrogated in the regions defined by the shared segments of interest. WES data was available on 964 controls [30] and 1,064 MM or MGUS cases including: 28 MM from the 11 Utah HRPs; 70 MM and 79 MGUS from 57 densely clustered families (each containing at least 2 MM/MGUS); 154 sporadic or early-onset MM/MGUS cases; and 733 sporadic MM cases from dbGaP [29]. Of the 57 densely clustered MM/MGUS families, 37 were ascertained by INSERM, France (36 MM, 69 MGUS, 2 controls), 10 by Mayo Clinic, Minnesota (10 MM, 10 MGUS, 11 controls), 6 by Memorial Sloan Kettering Cancer Center, New York (14 MM, 0 MGUS, 0 controls), 3 by International Agency for Research on Cancer, France (8 MM, 0 MGUS, 0 controls), and 1 by Weill Cornell, New York (2 MM, 0 MGUS, 0 controls). Most of the families had both MM and MGUS cases (32 families total), while 13 families only had MGUS and 12 families only had MM cases sequenced. Nine families had at least one unaffected relative sequenced as a control. (See S2 Table.)

Joint calling analysis. To perform joint calling of all of the exome sequences, we utilized the calling pipeline developed at the Icahn School of Medicine at Mt. Sinai, based on GATK Best Practices [59]. Briefly, fastq files were aligned to genome build 37 using bwa version 0.7.8, indels were realigned using GATK, duplicates were removed using Picard MarkDuplicates, and base quality scores were recalibrated using GATK. HaplotypeCaller was then used to generate individual GVCF files for each individual, and GenotypeGVCFs was used to generate the final joint calling. The jointly-called VCF was annotated with SNPEff and loaded into a GEMINI database for easy of querying. Additional functional annotations available in the GEMINI suite include CADD, ANNOVAR, conservation, location, and if the variant was listed in OMIM.

Variant prioritization. A GEMINI query was developed to select variants with high or medium impact, AAF < 0.001, in the shared segments of interest, and shared by the MM cases is the discovery pedigree, or identify other sequence variants in candidate gene across collaborating and publicly available exomes.

Burden testing. Burden testing was performed on jointly called and processed WES from 1,064 MM/MGUS cases and 964 unaffected controls for the 22 genes in the GG-NER incision complex and 15 genes in the SWI/SNF chromatin remodeling complex. The GEMINI software [60] was used to perform a c-alpha test [61] with 1000 permutations. Only variants with AAF < 0.05 and high or moderate predicted impact were included in the analysis.

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FIGURES

Fig. 1. Adequacy of the gamma distribution. The gamma distribution provides an adequate fit for multiple types of pedigrees. For example, HRP 549917 has gamma shape = 4.4 and rate = 3.6 with good visual density (a) and CDF (b) fit, with lambda = 0.9. HRP 34955 has gamma shape = 2.8 and rate = 2.9 with good visual density (c) and CDF (d) fit, with lambda = 1.0.

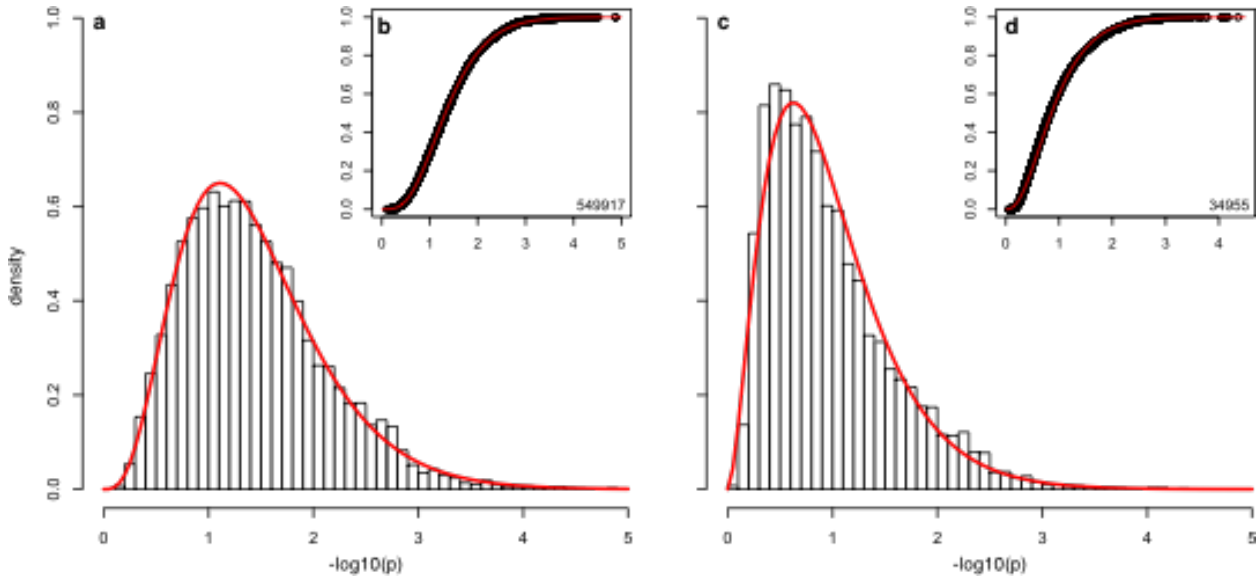
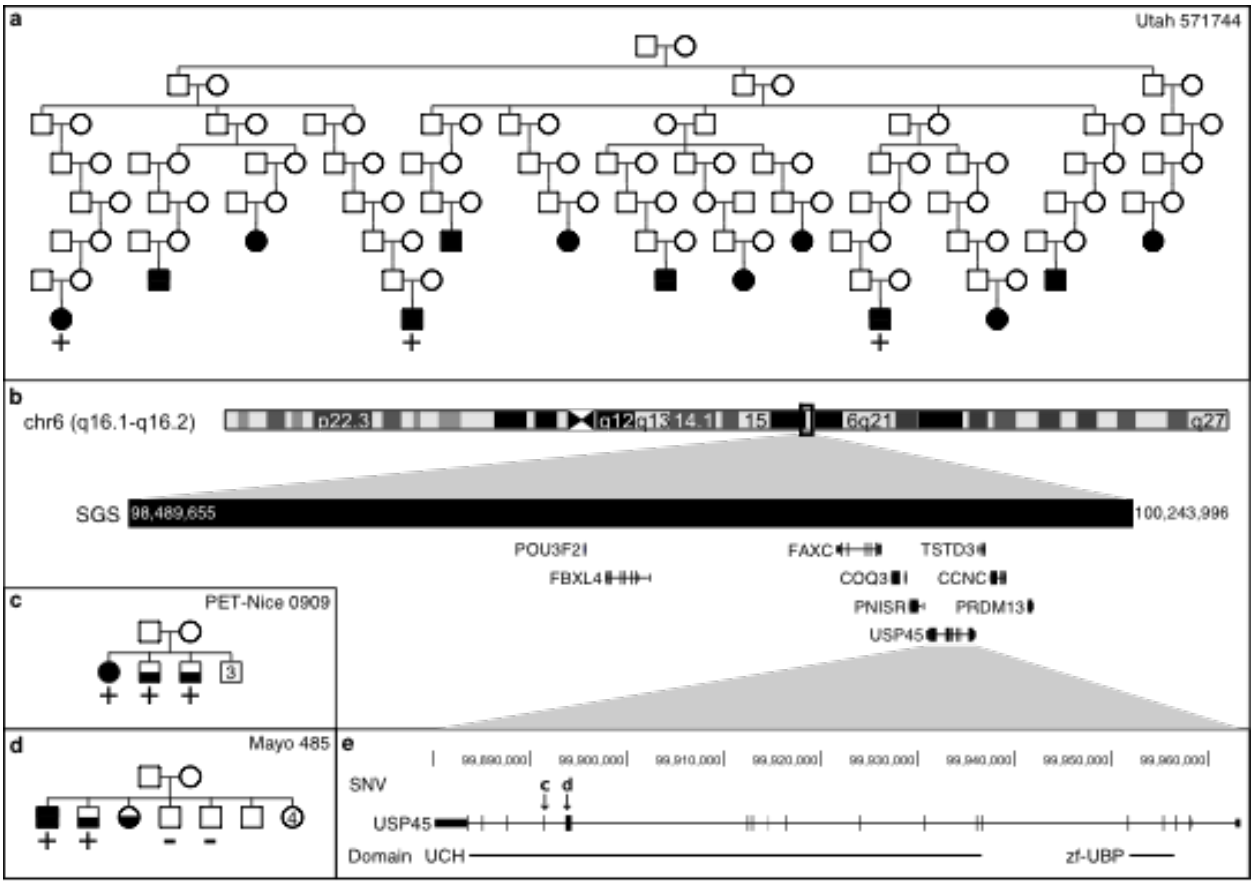
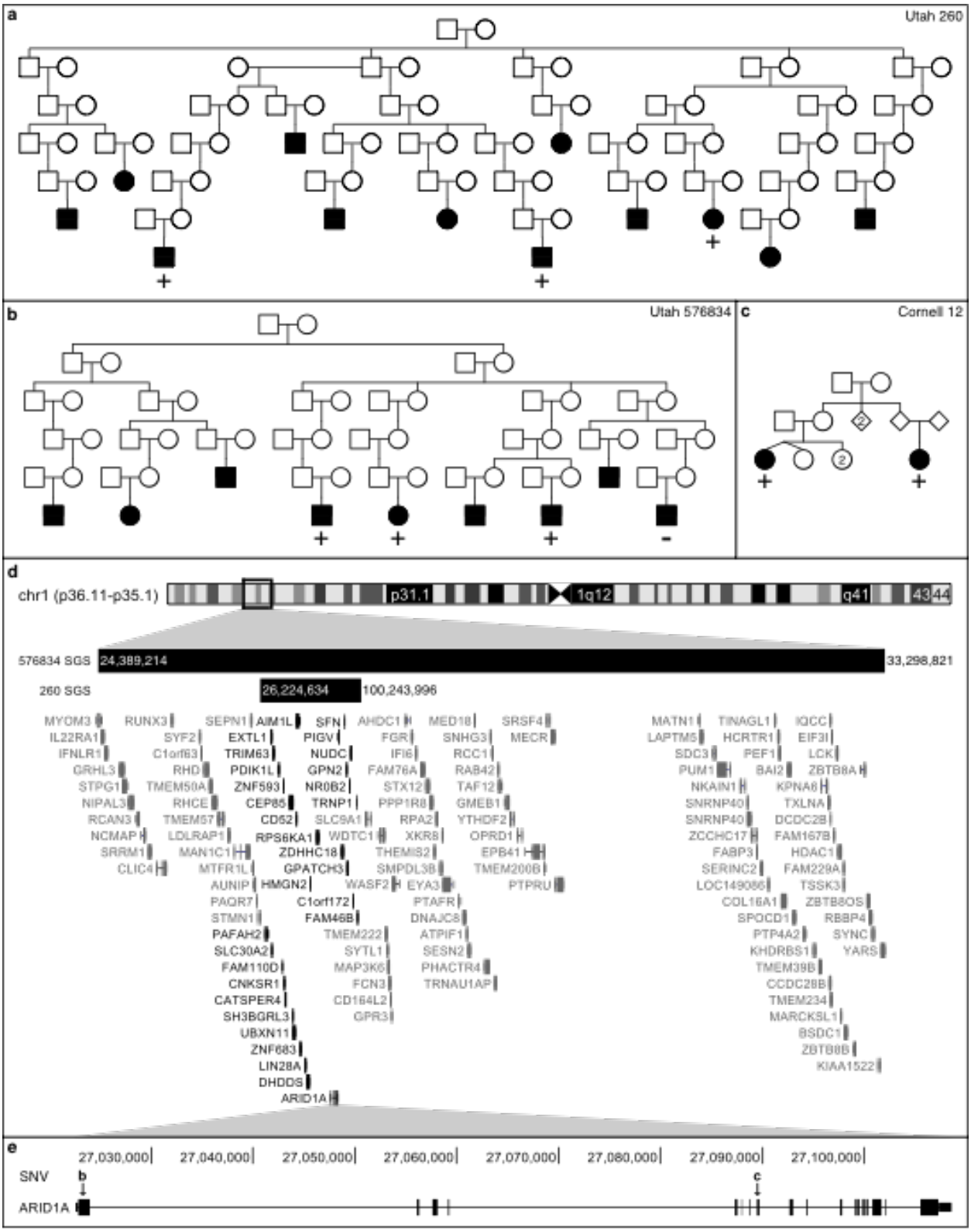


Fig. 2. Significant SGS, pedigrees, and segregating SNVs. In pedigrees, MM cases are fully shaded and MGUS cases are half shaded. Numbers indicate multiple individuals. a) Utah pedigree sharing the genome-wide significant SGS. The three genotyped MM cases that are SGS carriers are marked by +. Note. The genealogy extends beyond SEER cancer registry data. MGUS are unknown in this pedigree. b) Genomic region of significant SGS. c) INSERM pedigree carrying the stop gain SNV marked by “c” in box e. 1 MM and 2 MGUSs carry the SNV. d) Mayo Clinic pedigree carrying the missense SNV marked by “d” in box e. 1 MM and 1 MGUS carry the SNV, but not 2 unaffected siblings. e) Risk candidate gene, *USP45*, has 2 segregating SNVs in the ubiquitin C-terminal hydrolase 2 (UCH) domain.



1 **Fig. 3. SGS with multiple lines of evidence.** a/b) Utah pedigrees carrying the overlapping
2 SGSs on chr1p36.11-p35.1. + indicates the genotyped MM cases that are SGS carriers, -
3 indicates genotyped and non-carriers. c) Weill Cornell pedigree with a segregating, missense
4 SNV in *ARID1A* indicated by “c” in e. d) Genomic region of overlapping SGS. Dark black genes
5 fall in both regions. e) 2 rare and segregating, missense SNVs were observed in whole-exome
6 sequencing. SNV “b” is carried by the cases indicated with + in box b. SNV “c” in carried by the
7 cases in box c.

1


a
Utah 260
b
Utah 576834
c
Cornell 12
d
chr1 (p36.11-p35.1)
p31.1
1q12
q41
43
44
576834 SGS
24,389,214
33,298,821
260 SGS
26,224,634
100,243,996
MYOM3
RUNX3
SEPN1
AIM1L
SFN
AHDC1
MED18
SRSF4
IL22RA1
SYF2
EXTL1
PIGV
FGR
SNHG3
MECR
IFNLR1
C1orf63
TRIM63
NUDC
IFI6
RCC1
GRHL3
RHD
PDIK1L
GPN2
FAM76A
RAB42
STPG1
TMEM50A
ZNF593
NR0B2
STX12
TAF12
NIPAL3
RHCE
CEP85
TRNP1
PPP1R8
GMEB1
RCAN3
TMEM57
CD52
SLC9A1
RPA2
YTHDF2
NCMAP
LDLRAP1
RPS6KA1
WDTC1
XKR8
OPRD1
SRRM1
MAN1C1
ZDHHC18
THEMIS2
EPB41
CLIC4
MTFR1L
GPATCH3
SMPDL3B
TMEM200B
AUNIP
HMGN2
WASF2
EYA3
PTPRU
PAQR7
C1orf172
PTAFR
STMN1
FAM46B
DNAJC8
PAFAH2
TMEM222
ATPIF1
SLC30A2
SYTL1
SESN2
FAM110D
MAP3K6
PHACTR4
CNKSR1
FCN3
TRNAU1AP
CATSPER4
CD164L2
SH3BGRL3
GPR3
UBXN11
ZNF683
LIN28A
DHDDS
ARID1A
MATN1
TINAGL1
IQCC
LAPTM5
HCRTR1
EIF3I
SDC3
PEF1
LCK
PUM1
BAI2
ZBTB8A
NKAIN1
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SNRNP40
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SNRNP40
DCDC2B
ZCCHC17
FAM167B
FABP3
HDAC1
SERINC2
FAM229A
LOC149086
TSSK3
COL16A1
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SPOCD1
RBBP4
PTP4A2
SYNC
KHDRBS1
YARS
TMEM39B
CCDC28B
TMEM234
MARCKSL1
BSDC1
ZBTB8B
KIAA1522
e
27,030,000
27,040,000
27,050,000
27,060,000
27,070,000
27,080,000
27,090,000
27,100,000
SNV
b
c
ARID1A