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Critical assessment of candidate gene prioritization methods

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ABSTRACT

Motivation: Gene prioritization aims at identifying the most promising candidate genes among a large pool of candidates—so as to maximize the yield and biological relevance of further downstream validation experiments and functional studies. During the past few years, several gene prioritization methods have been defined and some of them have been implemented and made available through freely available web tools. In this study, we aim at comparing the predictive performance of eight publicly available prioritization methods on novel data. We have performed an analysis in which 42 recently reported disease gene associations from literature are used to benchmark these tools before the underlying databases are updated. Our approach mimics a novel discovery, and therefore the estimation of the performance is more realistic than when benchmarking through cross-validation on retrospective data.

Results: Our benchmark indicates that although the observed performance is slightly lower than for benchmarks on retrospective data, several methods can still efficiently identify the novel disease genes. There are however marked differences, and methods that rely on more advanced data integration schemes appear more powerful.

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1 INTRODUCTION

A major challenge in human genetics is to discover novel disease causing genes, both for Mendelian and complex disorders. Identifying disease genes is a crucial first step in unraveling molecular networks underlying diseases, and thus understanding disease mechanisms, also towards the development of effective therapies. The discovery of a novel disease gene often starts with a cytogenetic study, a linkage analysis, a high-throughput *omics* experiment, or a genome-wide association studies (GWAS). However, these studies do not always pinpoint the disease gene uniquely, but often result in large lists of candidate genes that are potentially relevant (Hardy and Singleton, 2009). Moreover, recent advances in next-generation sequencing offer promising opportunities to explore the genomic alterations of patients (Schuster, 2008). However, thousands of mutations in hundreds of genes are often detected, among which only a few are in fact linked to the genetic condition of interest (Lupski *et al.*, 2010). The experimental validation of these candidate genes, for instance through resequencing, pathway or expression analysis, is still expensive and time consuming. An efficient way to reduce the validation cost is to narrow down the large list of candidate genes to a small and manageable set of highly promising genes, a process called gene prioritization. Prioritization was in the past achieved manually by geneticists and biologists and was mainly based on their own expertise. Nowadays, biologists and geneticists can use computational approaches that can handle and analyze the large amount of genomic data currently available.

In the past few years, many gene prioritization methods have been proposed, some of which have been implemented into publicly available tools that users can freely access and use. We have recently reviewed web-based gene prioritization applications and described how they differ by the inputs they require, the outputs they produce, and the data they use (Tranchevent *et al.*, 2010). This information is summarized at our Gene Prioritization Portal (http://www.esat.kuleuven.be/gpp), that currently describes 33 prioritization methods. This web site has been designed to help researchers to carefully select the tools that best correspond to their needs. For instance, only few tools can prioritize the whole genome, which can be necessary when no positive regions can be identified beforehand, or when selecting candidates for a medium-throughput screen (instead of low-throughput validation). Another example is the study of a poorly characterized disorder, for which a prioritization method that do not rely on a set of known disease genes might be more suited.

However, beyond these conceptual differences, one essential parameter to consider when selecting gene prioritization methods is

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their respective performance—that is, their ability to identify novel disease genes among large lists of candidate genes. A common standard in bioinformatics is to estimate the performance with a benchmark analysis. Several publications that introduce a novel prioritization approach also describe a comparative benchmark with several existing methods (Hutz *et al.*, 2008; Köhler *et al.*, 2008; Thornblad *et al.*, 2007). However, these benchmarks are most of the time cross-validations of gold-standard disease data sets (*e.g.*, known data). Therefore, the estimation of the performance is likely an overestimate of the real performance (*i.e.*, on novel data) (Myers *et al.*, 2006). In particular, because some methods combine multiple data sources and their benchmarking suffers from what can casually be called the "everything-but-the-kitchen-sink" bias. Because different types of data are dependent on each other (for example, GO annotation, KEGG pathway membership, and MEDLINE abstracts) it becomes impossible to remove all cross-talk effects between data sources (e.g., removing MEDLINE data does not remove all information from the biomedical literature since much of it is present in GO and KEGG) to prevent contamination of the prediction of the disease gene by actual retrospective knowledge of this association. This makes it challenging to create benchmarks on retrospective data that are indicative of the performance of the method in an actual research setting. Next to benchmarking, some studies combine predictions made by several prioritization methods when analyzing disease associated loci, mostly for type 2 diabetes and obesity (Elbers *et al.*, 2007; Teber *et al.*, 2009; Tiffin *et al.*, 2006). However, the results have not been experimentally validated, which means that it is not possible to identify which methods made better predictions. Also, a few studies combine computational and experimental analysis: *in silico* generated hypothesis are then validated *in vivo*. We have, for instance, performed a computationally-supported genetic screen in *Drosophila* that led to the identification of 12 novel atonal genetic interactors (Aerts *et al.*, 2009). Although useful, such studies often rely on the use of a single tool and therefore cannot be used to compare different approaches. They also give no indication of the performance of the method in general, but only illustrate it on a single well-validated case.

In this study, we aim at comparing the performance of several freely accessible web-based gene prioritization methods on novel data, which, to our knowledge, has never been performed before. To this aim, we have designed a workflow in the spirit of the Critical Assessment of protein Structure Prediction (CASP) concept. In the case of CASP, a protein 3D structure is known but kept secret while dedicated algorithms predict the structure of that protein. These predictions are then compared to the real structure, and algorithms can therefore be compared. In our case, we select recently reported disease gene associations from literature and use several gene prioritization tools to make predictions immediately after publication (typically within two days). Our approach relies on the fact that, when the prioritization tools are used, the novel disease gene association of interest is not yet included in the databases that underlie these tools. As a consequence, our approach mimics a novel discovery, and therefore the estimation of the performance is more accurate.

2 METHODS

2.1 Gene prioritization methods

We aim at comparing the gene prioritization tools that can easily be used, and therefore only select the tools for which a free web-based implementation is available. The main objective is to assess the ability of the gene prioritization methods to discover novel disease gene. We have therefore not selected the methods whose ranking strategies depend exclusively on text mining (Vooren *et al.*, 2007; Hristovski *et al.*, 2005; Yu *et al.*, 2008; Xiong *et al.*, 2008) as they would most likely work only when the novel disease gene was already considered a good candidate gene prior to discovery. One exception is Candid that also uses other data sources beside MEDLINE (*e.g.*, protein domain, interactions, expression data). In total, we have selected eight methods: Suspects (Adie *et al.*, 2006), ToppGene (Chen *et al.*, 2007), GeneDistiller (Seelow *et al.*, 2008), GeneWanderer (Köhler *et al.*, 2008), Posmed (Yoshida *et al.*, 2009), Candid (Hutz *et al.*, 2008), Endeavour (Aerts *et al.*, 2006), and Pinta (Nitsch *et al.*, 2010). Originally, Pinta was developed to use expression data as input data, but here, we replace the continuous data (coming from expression data) with binary data using training genes: a *1* is inputed for each training gene, and a *0* is associated to the other genes. For an overview of the methods, please see Supplementary Table S1. All methods except Candid are used to prioritize a set of candidate genes (from a chromosomal region), and Candid is used to prioritize the whole genome. Pinta and Endeavour support both genome-wide and candidate set based prioritizations, and are used for both in this study (Endeavour-GW and Pinta-GW for genome-wide prioritization, Endeavour-CS and Pinta-CS for the candidate set prioritization). In addition, GeneWanderer can be run with up to four different ranking strategies (random walk, diffusion kernel, shortest path and direct interaction). We present the results for the first two strategies (GeneWanderer-RW for random walk, GeneWanderer-DK for diffusion kernel) since they have been showed to outperform the other two, simpler, methods (Köhler *et al.*, 2008) and since they can be efficiently used with many training genes. The performance of Posmed shows a strong dependency on the set of keywords used as an input and we run it twice with different inputs. In the first run, we use the complete keyword set (Posmed-KS), and in the second, we only use the name of the disease (Posmed-DN). GeneDistiller is trained with both genes and keywords. These keywords are then used to find additional genes through the mining of OMIM, which in our case has less influence since OMIM is already used to derive the training genes. We therefore consider that GeneDistiller is trained with genes only. Candid is the only method that can also be trained with disease specific tissues, when available, tissues relevant to the disease under study are used. The methods are run with the default options, in particular no fine tuning of the parameter is done. Notice that Suspects went offline during our study after the 27th association and is not supported anymore (Euan Adie, personal communication), therefore, Suspects results are based on 27 associations over 42.

2.2 Validation data set

The validation data set is built by mining the scientific literature to identify the recently discovered disease-gene associations. This is achieved manually to avoid false positive associations. We select 6 journals that frequently publish papers that describe such associations: Nature Genetics, American Journal of Medical Genetics (part A / part B), Human Genetics, Human Molecular Genetics, and Human Mutation. We select all the novel disease-gene associations regardless of the disease under study, of the methodology used, and of whether the findings are confirmed or not. Novelty is assessed by using OMIM (McKusick, 1998), the Genetic Association Database (Becker *et al.*, 2004), GoPubmed (Doms and Schroeder, 2005), and GeneCards (Safran *et al.*, 2010). More precisely, we assess novelty at the gene level, and therefore novel mutations within already known genes are not considered. This process was kept active for 6 months (May 15 - November 15, 2010) and led to a collection of 42 associations (see Table 1 and Supplementary Table S2). For each association, the methods are run as

soon as the association is identified following the defined workflow (see below). By doing this, we simulate as much as possible the prediction of a novel disease gene since the underlying databases are still unaware of the association.

Once an association is identified, the exact inputs for the different methods have to be defined. For instance, ToppGene, GeneDistiller, GeneWanderer, Pinta and Endeavour require training genes (genes already known to be associated to the disease under study) whereas Suspects, Posmed, GeneDistiller and Candid require keywords that describe the disease. Training genes and keywords are collected from the corresponding OMIM pages, GAD pages and from recently published reviews when possible. BioMart (Haider *et al.*, 2009) is used to map between gene symbols and method specific gene identifiers (*e.g.*, EntrezGene or Ensembl identifiers). As mentioned above, most of the methods require in addition a set of candidate genes (from the whole genome). Several methods accept chromosomal coordinates whereas some prefer cytogenetics bands. For each association, we select the cytogenetics bands that cover approximately 10Mb around the novel disease gene and derive the chromosomal coordinates. We choose 10Mb to obtain on average at least 100 candidate genes. Once again, BioMart is used to retrieve specific gene identifiers. For an overview of the inputs for the 42 associations, please see Supplementary Table S3.

2.3 Statistical measures

For each method, we then assess its ability to discover novel disease genes using several statistical measures. We first compute the median of the rank ratio over all associations. We preferably use rank ratio over rank because methods do not necessarily return the same number of candidate genes even when fed with the same inputs. In addition, we also draw the boxplots of these rank ratios to give a more comprehensive view of the method performance. Another efficient method to compare the methods is to build the Receiver Operating Characteristic (ROC) curves, and to compute the Area Under the Curve (AUC) as an estimate of the global performance. To compare the methods even further, we computed the true positive rates when setting the threshold for validation at the top 10% (TPR in top 10% of candidates) and 30% (TPR in top 30%). This is motivated by the fact that in a real situation, the number of candidate genes to assay often needs to be limited because of financial and time constraints. We have selected two thresholds that represent reasonable biological hypotheses, as we previously illustrated in a genetic screen (Aerts *et al.*, 2009). The corresponding TPR measures are used to estimate how efficient the methods are if only the top 10% or 30% candidate genes would be assayed. There are cases for which some methods are not able to identify the novel disease gene at all, we therefore include a reliability measure. It is defined as the percentage of associations for which each method does return a prioritization result for the novel disease gene (in some cases a method will not return any result, for example because it could not correctly map the gene identifier or some candidates are otherwise filtered out).

These five measures are then summarized as pentagons in Figure 3, the bigger the pentagon the better (the origin represents the worse case). Lastly, we also derive a heat map to detect any correlation between methods by computing the pairwise cosine similarity of the rankings presented in Tables 2 and 3 (see Supplementary Figure S1).

3 RESULTS

The overall ranking results of all gene prioritization methods are presented in Tables 2 and 3 for the candidate gene set based and genome-wide methods respectively. In addition, Figure 3 summarizes graphically all performance measures for all methods. These results have also been added to the Gene Prioritization Portal (http://www.esat.kuleuven.be/gpp).

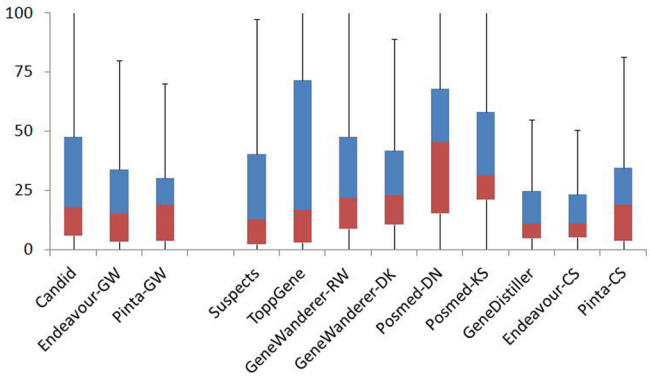


Figure 1. Ranking results of the novel disease genes from the validation data set illustrated as boxplots for the genome-wide (left) and candidate gene set based (right) prioritization methods.

3.1 Rank Ratio

When considering the median of the rank ratios, GeneDistiller, Endeavour-CS, and Suspects are the methods that perform the best (respectively 11.11, 11.16, and 12.77). They are followed by Endeavour-GW (15.49), ToppGene (16.8), Candid (18.1), Pinta-CS (18.87), Pinta-GW (19.03), GeneWanderer-RW (22.11), GeneWanderer-DK (22.97), Posmed-KS (31.44), and Posmed-DN (45.45). The boxplots presented in Figure 1 illustrate that both, GeneDistiller and Endeavour-CS perform better than the other candidate set based prioritization methods (Figure 1-right). Among the genome-wide methods, Endeavour-GW performs slightly better than Pinta-GW and Candid (Figure 1-left).

3.2 Reliability

The reliability measure represents the percentage of associations for which each method is able to prioritize the novel disease gene. When considering reliability, Endeavour (both modes), Candid, and Pinta (both modes) performed the best with 100% closely followed by ToppGene, GeneDistiller, and GeneWanderer-RW with more than 95% (meaning that only one or two associations are missing). At the other hand of the spectrum, Posmed-KS and Posmed-DN only work for about half of the experiments (respectively 47.6% and 50%).

3.3 Area Under the Curve

When we compare the methods based on the global AUC (see Figure 2), we observe that GeneDistiller appears as the best performing method overall with an AUC of 86%. It is followed by Endeavour-CS (82%), Endeavour-GW (79%), Pinta-GW (77%), Suspects (76%), Pinta-CS (75%), Candid (73%), GeneWanderer-RW (71%), GeneWanderer-DK (67%), ToppGene (66%), Posmed-KS (58%), and Posmed-DN (56%). The ROC curves are in general intertwined meaning that none of the approaches is clearly performing better than the other. However, we postulate that, in our case, the most important section of the ROC curve is the beginning and therefore use two other measures, the true positive rates at 10% and at 30%.

Table 1. The validation data set consisting of 42 recently discovered disease gene associations

Gene	Disease / phenotype	Reference(s)
HCCS	Congenital Diaphragmatic Hernia	Qidwai *et al.* (2010)
BRCA2	Bipolar Disorder	Tesli *et al.* (2010)
TNFRSF19	Nasopharyngeal carcinoma	Bei *et al.* (2010)
MECOM	Nasopharyngeal carcinoma	Bei *et al.* (2010)
ATF7IP	Testicular germ cell tumor	Turnbull *et al.* (2010)
DMRT1	Testicular germ cell tumor	Turnbull *et al.* (2010)
FUT2	Crohn's disease	McGovern *et al.* (2010)
CSF1R	Asthma	Shin *et al.* (2010)
GLI3	Metopic craniosynostosis	McDonald-McGinn *et al.* (2010)
STOM	Nonsyndromic cleft lip/palate	Letra *et al.* (2010)
UTRN	Arthrogryposis	Tabet *et al.* (2010)
GABRR1	Bipolar schizoaffective disorder	Green *et al.* (2010)
UBE2L3	Crohn's disease	Fransen *et al.* (2010)
BCL3	Crohn's disease	Fransen *et al.* (2010)
EZH2	Myelodysplastic syndromes	Nikoloski *et al.* (2010)
TRAF6	Parkinson's disease	Zucchelli *et al.* (2010)
IL10	Behçet's disease	Remmers *et al.* (2010); Mizuki *et al.* (2010)
DAB2IP	Abdominal aortic aneurysm	Gretarsdottir *et al.* (2010)
SPIB	Primary biliary cirrhosis	Liu *et al.* (2010)
MMEL1	Primary biliary cirrhosis	Hirschfield *et al.* (2010)
TBX2	Complex heart defect	Radio *et al.* (2010)
RUNX2	Single-suture craniosynostosis	Mefford *et al.* (2010)
CRHR1	Multiple sclerosis	Briggs *et al.* (2010)
IFNG	Leprosy	Cardoso *et al.* (2010)
SH2B1	Congenital Anomalies of the Kidney and Urinary Tract	Sampson *et al.* (2010)
DISP1	Congenital Diaphragmatic Hernia	Kantarci *et al.* (2010)
G6PC3	Dursun syndrome	Banka *et al.* (2010)
PQBP1	Periventricular heterotopia	Sheen *et al.* (2010)
CD320	Methylmalonic aciduria	Quadros *et al.* (2010)
CHST14	Ehlers-Danlos syndrome	Miyake *et al.* (2010)
PLCE1	Esophageal squamous cell carcinoma	Wang *et al.* (2010); Abnet *et al.* (2010)
C20orf54	Esophageal squamous cell carcinoma	Wang *et al.* (2010)
SDCCAG8	Retinal-renal ciliopathy	Otto *et al.* (2010)
TP63	Lung adenocarcinoma	Miki *et al.* (2010)
UBE2E2	Type 2 diabetes	Yamauchi *et al.* (2010)
LPP	Tetralogy of Fallot	Arrington *et al.* (2010)
RANBP1	Smooth pursuit eye movement abnormality	Cheong *et al.* (2011)
HTR7	Alcohol dependence	Zlojutro *et al.* (2010)
SOX17	Congenital anomalies of the kidney and the urinary tract	Gimelli *et al.* (2010)
ACAD9	Mitochondrial complex I deficiency	Haack *et al.* (2010)
TRAF3IP2	Psoriasis	Ellinghaus *et al.* (2010); Hüffmeier *et al.* (2010)
WDR62	Autosomal recessive primary microcephaly	Yu *et al.* (2010); Nicholas *et al.* (2010)

3.4 True positive rates

Considering the TPR in top 10% and 30%, we can observe a similar trend. Indeed, at 10%, GeneDistiller is first with a rate of 47.6% (20 associations found over 42), followed by both ToppGene and Endeavour-CS with 42.9% (18 associations). However, at 30%, the best method is Endeavour-CS (90.5% - 38 associations), followed by GeneDistiller (78.6% - 33 associations). The other methods show smaller TPR at both levels: Pinta-CS (31%,71.4%), Suspects (33.3%, 63%), GeneWanderer-RW (26.2%, 61.9%), GeneWanderer-DK (21.4%, 52.4%), Posmed-KS (7.1%, 23.8%), and Posmed-DN (11.9%, 23.8%). Among the genome-wide prioritization methods, Endeavour-GW shows highest TPR in top 10% and 30% (38.1%, 71.4%), followed by Candid (33.3%, 64.3%) and Pinta-GW (31%, 71.4%).

3.5 Correlations

Supplementary Figure S1 shows the heat map of the novel disease gene ranking positions for all methods in this study. For the methods that have two modes (*i.e.*, Posmed, GeneWanderer, Endeavour, Pinta), the two modes are highly correlated ($\geqslant 0.89$). There is also a significant correlation between Candid and GeneWanderer-DK (0.82). The other values are within 0.4 and 0.7, indicating that all methods are moderately correlated.

Table 2. Ranking positions (as rank ratios) of the 42 novel disease genes from the validation data set for the candidate set based prioritization methods. (*) Values computed only on the first 27 associations.

Gene	ToppGene		GeneWanderer-DK		Posmed-KS		Endeavour-CS			Median
	Suspects		GeneWanderer-RW		Posmed-DN		GeneDistiller		Pinta-CS	
HCCS	23.08	3.39	46.81	37.5	15.38	n.a.	7.69	8.89	15.69	15.54
BRCA2	63.64	2.13	9.68	8.33	30	37.5	1.28	2.9	2.86	8.33
TNFRSF19	14.12	13.04	31.48	41.86	7.69	28.13	31.29	22.76	26.23	26.23
MECOM	n.a.	n.a.	45	11.11	n.a.	75	24.34	8.06	26.92	25.63
ATF7IP	69.9	37.44	76.47	39.52	n.a.	n.a.	11.39	41.73	66.88	41.73
DMRT1	0.97	71.7	28.57	n.a.	21.43	21.43	15.79	97.78	37.5	25
FUT2	10.48	89.71	50	92.09	n.a.	n.a.	26.19	27.41	17.11	27.41
CSF1R	0.94	1.6	6.35	3.77	45.45	15.56	1.12	5	9.76	5
GLI3	22.06	1.12	2	2.44	50	33.33	0.85	3.85	1.92	2.44
STOM	17.65	67.37	6.85	4.69	n.a.	n.a.	35.65	12.71	15.79	15.79
UTRN	2.5	2.94	12.5	10.53	20	n.a.	5	2.04	3.33	4.17
GABRR1	11.43	5.26	56	54.55	85.71	100	4.55	25.76	12.9	25.76
UBE2L3	79.71	87.35	16.83	16.47	n.a.	n.a.	9.14	28.99	2.68	16.83
BCL3	0.84	2.17	3.65	11.17	n.a.	n.a.	5.78	6.52	10.58	5.78
EZH2	37.37	77.01	18.37	36.59	60.8	21.11	8.05	16.06	20.63	21.11
TRAF6	4.48	40.85	6.52	11.63	4.76	23.53	0.82	27.55	4.84	6.52
IL10	1.81	1.35	0.87	27.55	2.7	2.56	8.44	28.5	0.50	2.56
DAB2IP	49.28	91.25	22	30.23	n.a.	n.a.	37.7	20.34	20.78	30.23
SPIB	71.88	16.8	24.73	12.58	94	82.35	6.37	7.69	35.89	24.73
MMEL1	4.29	58.74	n.a.	n.a.	n.a.	n.a.	51.71	22.68	24.09	24.09
TBX2	n.a.	1.11	n.a.	n.a.	n.a.	n.a.	11.3	0.51	2.86	1.98
RUNX2	2.34	1.65	1.14	2.94	9.52	4.26	1.31	2.01	0.99	2.01
CRHR1	10.56	3.48	23.58	24.14	21.82	21.31	17.65	10.22	15.18	17.65
IFNG	1.56	1.64	2.04	7.89	5.88	10	0.91	2.94	1.92	2.04
SH2B1	n.a.	80	53.09	20.55	n.a.	n.a.	7.47	11.81	13	16.77
DISP1	67.74	10.87	11.54	n.a.	n.a.	n.a.	8.11	22.22	96.67	16.88
G6PC3	22.76	23.86	40.37	n.a.	n.a.	n.a.	11.99	19.12	59.39	23.31
PQBP1	offline	6.54	15.22	22.97	n.a.	n.a.	1.14	8.55	38.24	11.88
CD320	offline	51.59	74	85.88	n.a.	n.a.	24	23.68	25	38.3
CHST14	offline	30.11	20	97.18	100	100	25	7.65	22.22	27.55
PLCE1	offline	3.23	22.22	35.29	60	29.55	27.07	11.11	50	28.31
C20orf54	offline	36.77	98.88	95.89	n.a.	n.a.	n.a.	97.19	94.06	95.89
SDCCAG8	offline	1.64	63.64	93.1	n.a.	n.a.	40	1.3	2.44	21.22
TP63	offline	3.23	14.29	15.15	n.a.	n.a.	0.89	1.82	11.63	7.43
UBE2E2	offline	94.87	51.85	52.17	n.a.	n.a.	41.56	27.42	75.76	52.01
LPP	offline	85.37	61.4	78.26	87.5	40	11.11	11.21	22.73	50.7
RANBP1	offline	93.39	28.17	23.73	n.a.	n.a.	18.31	57.93	40.77	34.47
HTR7	offline	82.56	1.75	6.38	n.a.	n.a.	0.85	0.89	3.23	2.49
SOX17	offline	31.18	2.56	20.59	60	48	5.04	10.2	3.13	15.4
ACAD9	offline	1.39	10.89	2.3	68	95.16	31.5	1.58	31.30	21.1
TRAF3IP2	offline	8.47	25	6.45	90.91	52.63	14.29	19.33	28.26	22.16
WDR62	offline	91.54	95.71	86.67	n.a.	n.a.	37.43	7.14	63.95	75.31
Median	**12.77***	**16.8**	**22.11**	**22.97**	**45.45**	**31.44**	**11.11**	**11.16**	**18.87**	
Reliability	**88.9%***	**97.6%**	**95.2%**	**88.1%**	**50%**	**47.6%**	**97.6%**	**100%**	**100%**	
TPR in top 10%	**33.3%***	**42.9%**	**26.2%**	**21.4%**	**11.9%**	**7.1%**	**47.6%**	**42.9%**	**31%**	
TPR in top 30%	**63.0%***	**52.4%**	**61.9%**	**52.4%**	**23.8%**	**23.8%**	**78.6%**	**90.5%**	**71.4%**	

4 DISCUSSION

We aim at assessing the usefulness of eight gene prioritization methods that are freely available via web applications. We have built a validation scheme in the spirit of the CASP scheme for protein structure prediction. The validation is based on 42 recently discovered disease-gene associations from literature and contains novel genes for both monogenic conditions and complex disorders. We have selected novel disease-gene associations regardless of their strength, and of the underlying methodology. To mimic a real discovery, we have run the methods as soon as the article appeared online so that all databases used for gene prioritization are still not contaminated by the knowledge of the novel disease-gene association. This also means that we had to exclude methods that query MEDLINE online

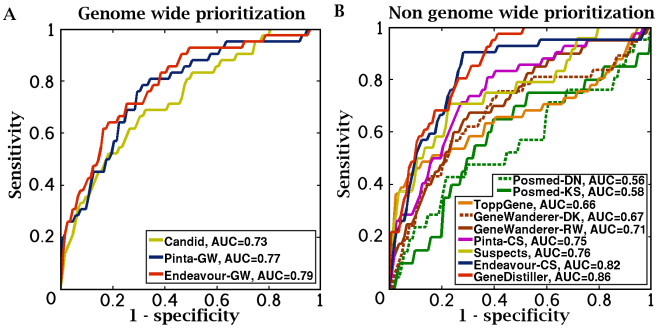


Figure 2. ROC curves of the genome-wide (A) and candidate gene set based (B) prioritization methods.

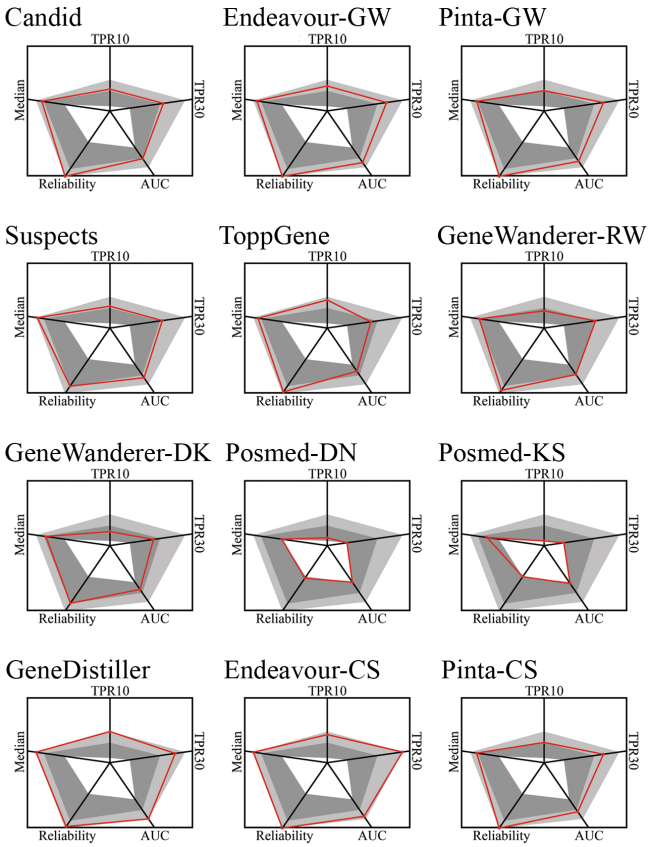


Figure 3. Summary of the five performance measures per gene prioritization method. The red pentagons represent the performance of the methods. In addition, on each axis, the maximum, the average and the minimum values are displayed (respectively in light grey, dark grey and white).

Table 3. Ranking positions (as rank ratios) of the 42 novel disease genes from the validation data set for the genome-wide gene prioritization methods.

Genes	Candid	Endeavour-GW	Pinta-GW	Median
HCCS	25.85	2.75	10.78	10.78
BRCA2	1.37	1.25	0.29	1.25
TNFRSF19	5.74	36.09	21.24	21.24
MECOM	11.6	15.89	30.24	15.89
ATF7IP	1.38	49.88	58.21	49.88
DMRT1	41.83	79.04	29.94	41.83
FUT2	47.67	42.43	19.74	42.43
CSF1R	2.93	1.67	2.69	2.69
GLI3	7.92	2.25	0.76	2.25
STOM	26.54	6.52	22.72	22.72
UTRN	18.51	0.46	2.99	2.99
GABRR1	17.43	15.97	11.75	15.97
UBE2L3	49.17	71.21	6.34	49.17
BCL3	60.27	4.74	10.76	10.76
EZH2	4.66	34.43	23.41	23.41
TRAF6	0.13	16.54	8.11	8.11
IL10	13.22	26.26	0.18	13.22
DAB2IP	14.2	26.4	21.03	21.03
SPIB	5.56	10.28	30.44	10.28
MMEL1	25.2	39.37	18.32	25.2
TBX2	31.34	1.87	1.34	1.87
RUNX2	1.79	3.07	0.18	1.79
CRHR1	15.04	13.35	12.94	13.35
IFNG	47.19	1.21	0.21	1.21
SH2B1	3.53	10.82	12.64	10.82
DISP1	2.26	32.85	93.23	32.85
G6PC3	47.72	18.72	51.22	47.72
PQBP1	22.51	16.74	34.79	22.51
CD320	80.54	46.95	26.41	46.95
CHST14	61.64	7.57	25.8	25.8
PLCE1	10.45	13.03	42.6	13.03
C20orf54	67.5	95.54	95.04	95.04
SDCCAG8	78.11	5.23	0.85	5.23
TP63	7.04	1.35	11.67	7.04
UBE2E2	51.18	17.38	61.89	51.18
LPP	74.55	6.93	17.62	17.62
RANBP1	7.32	46.07	48.2	46.07
HTR7	17.7	0.56	1.73	1.73
SOX17	74.67	15.08	1.11	15.08
ACAD9	33.45	1.53	31.53	31.53
TRAF3IP2	2.05	39.04	23.1	23.1
WDR62	29.98	23.35	63.97	29.98
Median	**18.1**	**15.49**	**19.03**	
Reliability	**100%**	**100%**	**100%**	
TPR in top 10%	**33.3%**	**38.1%**	**31%**	
TPR in top 30%	**64.3%**	**71.4%**	**71.4%**	

since their results would be biased.

We want to compare the performance of the methods even if the inputs are different (genes vs. keywords, genome-wide vs. candidate set). Among the eight gene prioritization methods that we have analyzed in this study, only Endeavour, Candid, and Pinta have been used for genome-wide prioritization. The input data for Endeavour and Pinta are training genes, whereas Candid requires keywords. The gene prioritization methods that we have used to prioritize candidate genes within a region of interest are Suspects, ToppGene, GeneWanderer, Posmed, GeneDistiller, and again Endeavour and Pinta. Suspects and Posmed are trained with keywords, the other methods require training genes. We have extensively searched through literature and dedicated databases to identify as many reliable training genes as possible for the disease of interest, as well

as a set of appropriate keywords to derive fair and meaningful comparisons. However, different, and possibly better, results might be obtained by tuning the parameters or by refining the inputs.

Our validation is too small to claim that the differences among the methods are significant. However, a trend can still be observed, GeneDistiller and Endeavour-CS consistently appear as the best methods when looking at all performance measures. It is interesting to notice that the best results are in general obtained with methods that use many data types in conjunction (up to eight for Endeavour, as compared to the three data sources used by Posmed), but there is no perfect correlation. This is in agreement with the conclusion of the recent review by Tiffin *et al.* (2009), who indicate that successful computational applications will be facilitated by improved data integration.

All methods except Posmed have a high reliability measure ranging from 88% to 100%, meaning that at least 37 of the 42 novel disease genes are prioritized (or 24 of 27 for Suspects). However, the reliabilities for Posmed-KS and Posmed-DN are respectively 47.6% and 50%, which can be explained by the fact that Posmed also acts as a filter on the candidate genes to obtain a reduced list of genes in the end. There are therefore cases for which the novel disease gene has been removed by the filter. This is different from the other methods for which missing genes basically correspond to genes that are not recognized by the method (it happens most of the time with poorly characterized genes, such as C20orf54). Another special case is Suspects that went offline during the validation and therefore could only be validated with the first 27 associations. We therefore calculated the reliability only on the first 27 associations.

Two types of methods can be distinguished, the ones that are trained with already known genes and the ones that are trained with descriptive keywords. It appears that gene-based methods seem to work better than keyword-based methods (the average of medians is 17.2 for genes based methods and 27 for keyword based methods - similar results are obtained with the other measures, see Supplementary Table 8). This could be because we use in general more genes than keywords for training (18.8 genes on average for 6 keywords). This also indicates that more keywords might be needed to model a disease, a small text (such as an OMIM entry) might even be necessary (van Driel *et al.*, 2006).

There is in general an agreement between the four performance measures we use. One notable exception exists for ToppGene, whose AUC is 66%, and corresponds to rank 10^{th} (out of the 12 prioritization methods). In contrast its associated TPR in top 10% is 42.9%, which corresponds to rank 2^{nd}. This apparent contradiction can be explained by observing Figure 2, in which the ROC curve exhibits a non convex shape. This is because ToppGene either ranks the novel disease gene on top or at the bottom (*i.e.*, the disease genes are rarely ranked in the middle). And therefore the TPR in top 10% will be high because it only takes into account the top of the list, while the AUC will be lower because it basically behaves like an average over all cases. Another important point is that our observations are in line with the 'no free lunch' theorem. If we do not consider Posmed, each of the remaining seven methods can perform better than all the others for some cases, or, in other words, none of the seven methods outperforms another on the complete data set.

Posmed-KS has been trained with the complete keyword set, whereas Posmed-DN has been trained only with the disease name. The median rank ratio is 31.44 when the complete keyword set is used and drops to 45.45 when only the disease name is inputted. If we only compare the results over the 19 associations for which both methods are able to prioritize the novel disease gene, the difference becomes even larger (29.6 and 50 respectively for Posmed-KS and Posmed-DN). Altogether, these results indicate that Posmed does not rely on the use of the single disease name and that the extra keywords are important. It can be observed that the performance measures for Posmed are worse than for the other methods. However, when looking at the individual ranks, it can be observed that Posmed returns far fewer genes than the other methods because it also acts as a filter. As a result, the rank ratios are on general larger and the performance measures are therefore worse. As such, it becomes difficult to fairly compare Posmed to the other methods because our measures of performance naturally penalize the fact that Posmed returns prioritizations for a limited set of candidates. Changing our performance measures to counterbalance this effect would then give an unfair advantage to Posmed because it returns prioritizations only for the "safer bets".

GeneWanderer has also been run twice with different network algorithms: random walk (RW) and diffusion kernel (DK). The respective performance are very similar although the random walk approach is performing a little bit better than the diffusion kernel albeit non significant (22.11 to 22.97 for median rank ratio - similar differences are observed with the other measures). The heat map indicates a strong correlation ($>$0.9, see Supplementary Figure S1) between the two modes, which was expected since applying diffusion to a kernel can be interpreted as equivalent to applying a random walk on the underlying network. Altogether, this indicates that these two algorithms are similar.

Endeavour and Pinta are used to prioritize both the whole genome (Endeavour-GW and Pinta-GW) and the defined chromosomal region (Endeavour-CS and Pinta-CS) allowing us to identify the influence of the size of the gene list to prioritize. The median rank ratio is better for Endeavour-CS (11.16) than for Endeavour-GW (15.49). The difference is smaller but remains when considering the AUC, and the TPR in top 10% and 30%. The same training genes are used, and therefore the observed difference is only caused by extending the small candidate gene set to the whole genome. This confirms previous findings that prioritizing the whole genome is more difficult than prioritizing a rather small positive locus. The heat map indicates that the two Endeavour modes are strongly correlated as expected since the core algorithm is the same in both modes ($>$0.9, see Supplementary Figure S1). At contrary, the results for both Pinta modes are very similar (correlation of 0.99) and seem to indicate that the size of the candidate set does not influence this algorithm.

An important feature that might influence the results is the date of the last data update. The latest genomic data (still prior to discoveries considered in this study) is likely to give the best results since it will model more accurately what is currently known, when compared to data that is two year old. In our setup, we have no control over the genomic data used and can not identify if variation in performance among methods can be explained by this.

It is important to notice that the 42 novel disease gene associations do not represent a very homogeneous set. Indeed, the median of the rank ratios over the methods (rightmost column in Tables 3 and 2) show that some associations seem to be easier to predict than others. This also explains why all methods are moderately correlated on the heat map (> 0.4). A plausible explanation is the disparity in the available data between the novel disease genes. Since only

little data can be gathered for poorly characterized genes, such as C20orf54, they are more difficult to prioritize. However, we also hypothesize that the nature of the underlying genetic disorder, as well as the quality of the reported association might influence the ability of the methods to predict correctly that association. We have therefore divided the associations between confirmed (for monogenic diseases, the mutation is found in at least 2 unrelated patients; for multifactorial diseases, a GWAS is replicated in a separate cohort), intermediate (a single study, but additional functional evidence is provided), and unconfirmed (a single study). Among the 42 associations, 23 are confirmed, 8 are intermediate, and 11 are unconfirmed (see Supplementary Table S2). We hypothesize that this might influence our validation since some associations might in fact be spurious. However, we cannot observe any significant difference between the confirmed and unconfirmed associations in our case (see Supplementary Tables S4 and S5). Although this could be caused by the size of our validation data set, it may also indicate that the unconfirmed associations are of good quality.

In our validation data set, there are 17 monogenic diseases and 25 multifactorial disorders (see Supplementary Tables S6 and S7). It has been shown that it is more difficult to make predictions for multifactorial diseases than for monogenic diseases (Linghu *et al.*, 2009). Our results however seem to indicate that not all methods are influenced by the intrinsic complexity of multifactorial diseases. For instance, Endeavour, Candid and ToppGene seems to perform better for monogenic conditions while GeneWanderer, Suspects, and Posmed surprisingly perform better for complex disorders. However, the size of our validation data set does not allow for a complete statistical analysis. Larger validation data sets and real predictive studies will be pursued to complement our preliminary study.

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