1	

Types and prevalence of genetic disorders in humans	• Single gene disorders
	  ○ There are many, but most are very rare (<1 in 10,000)
	  ○ Total incidence in newborns is approx. 1 in 100
	  ○ Specific disorders may occur more frequently in certain populations
	  ○ Maintained due to heterozygote advantage or founder effect
	  ○ Most show recessive inheritance (one working copy is as good as two)
	  ○ Some are dominant due to:
	  ▪ Haploinsufficiency – loss of function in a dosage sensitive gene resulting in 50% of normal protein production not being enough to produce the wild type phenotype
	  ▪ Dominant-negative mutations – loss of function mutations in which the protein is made but not functional, and inhibits the function of the normal protein in heterozygotes
	  ▪ Gain of function mutations – new function of the gene product, or the protein is always active, or there are increased levels of expression, or inappropriate expression
	• Chromosomal
	  ○ Most caused by aneuploidy
	  ○ One chromosome is present more or less than normal
	  ○ More sever for larger, autosomal chromosomes
	  ○ Aneuploids very frequent among spontaneous miscarriages (40-50% in the first trimester)
	  ○ Chromosomal aberrations also occur – translocations, deletions, duplications
	• Multifactorial
	  ○ Part genetic, part environmental origin
	  ○ Genetic component is usually polygenic (<1 gene)
	  ○ Congenital abnormalities (e.g. spina bifida) or late onset (e.g. type I diabetes)

Gene structure and expression	• A gene is a sequence of DNA that is required for the production of a functional product – either a polypeptide or a functional RNA molecule • Include the coding sequence as well as adjacent sequences required for proper expression (e.g. promoters, terminators, regulatory sequences)
2	
Determining inheritance patterns	• Unaffected families who may carry the disease but have no affected children won't come to genetic centres, thus won't be included in data when analysed – thus may not show in a Mendelian ratio • Variable expressivity – may be difficult to assess the phenotype at the extreme closest to normal, especially for behavioural phenotypes (e.g. autism) • Variable penetrance – some individuals with the affected genotype do not show the phenotype • New mutations may arise • Locus heterogeneity – mutations in several different genes may show the same phenotype (often when mutations are in genes in biochemical pathways

Linkage analysis	• Linkage groups correspond with individual chromosomes • Genes may be mapped by following their joint segregation patterns in pedigrees • Can also be mapped by association with individual chromosomes in somatic cell hybrids between humans and another species (human chromosomes variably lost) • Can also be mapped by in situ hybridisation of a labelled antisense copy, binds to its locus in a chromosome spread • Requires allelic differences at two loci • Linkage phase Coupling: $\frac{A\ B}{a\ b}$ Repulsion: $\frac{A\ b}{a\ B}$ ○ • The chance of crossover occurring between two loci is proportional to their distances apart • Maximum value is 50% (above this and the markers are on different chromosomes)
LOD scores	• Allows sufficient data to be collected from pedigrees (won't be able to get enough offspring to analyse from one family when using human data) • Based on calculating the chance of getting a sibship assuming the two loci show a recombination fraction of r (range 0-0.5) ○ Divide this by the chance of getting the sibship assuming the two loci are unlinked (R=0.5) ○ This is the odds that the genes are linked (theta) ○ Take log(10) of this (Z), called the LOD score (log of the odds) ○ Repeat for all sibships ○ Then add the individual LOD scores (because they are logs, they can be added) • E.g. if 7 offspring all show parental inheritance **Pedigree 1:** **r** | **chance** $(1-r)^7$ | **odds (θ)** chance r/(chance r= 0.5) | **$\log_{10}$ odds (Z)** LOD **0.0** | **1.000** | **128** | **2.11** **0.1** | **0.478** | **61.2** | **1.79** **0.2** | **0.210** | **26.8** | **1.43** **0.3** | **0.082** | **10.5** | **1.02** **0.4** | **0.028** | **3.58** | **0.554** **0.5** | **0.0078** | **1.00** | **0.000** • Highest odds indicate the most likely • Z score must be higher than 3
3	
Identifying human disease genes	• Positional cloning ○ Identifying a gene after mapping it ○ The more extensive the mapping, the smaller the candidate region, the easier it is • Steps in positional cloning ○ Obtain the sequence of all the DNA in the candidate region ○ Identify all the genes in the region ○ Prioritise the genes for mutant screening (obtain candidate genes) ○ Test candidate genes for mutations in affected individuals

	○ Confirm through animal models • Obtaining the DNA sequence – chromosome walking ○ Genomic DNA subjected to partial restriction digest ○ Generates large fragments of overlapping DNA sequences ○ Cloned into a vector – makes a gneomic library ○ Start by identifying a clone that overlaps one of the markers that identify the candidate region ○ Use this first clone to probe the library and identify overlapping DNA fragments • Identifying all the genes in the region ○ Gene prediction – look for open reading frames ○ Zoo blots – probe southern blot of genomic DNA from other species with human probes (sequences part of genes are more likely to be conserved) ○ CpG islands – clusters of unmethylated CpG dinucleotides found near many transcription initiation sites ○ Exon trapping – clone random fragments from the region of interest into a special vector that is engineered so that a splicing reaction will occur if the cloned fragment contains and intron/exon boundary • Prioritise the genes to obtain candidate genes ○ Perform BLAST searches with predicted genes ○ Look for appropriate expression • Confirming the candidate gene ○ Mutation screening in affected individuals ○ Rescue the phenotype ○ Production of an animal model of the disease
4	
Human mutation	• Types of single gene mutations ○ Base substitution – single base change ▪ Transition – pyrimidine raplaced by another pyrimidine (C/T) or purine by purine (G/A) ▪ Transversion – purine replaced by pyrimidine (or vice versa) ○ Insertions or deletions – short DNA sequences may be deleted or added • Effects of single gene mutations on gene products ○ Within coding sequence ▪ Silent mutations – no alteration to amino acid sequence (production may be slowed down) ▪ Missense mutations – amino acid does change ▪ Nonsense mutation – codon changes to a stop codon ▪ Frameshift mutation – insertion or deletion of 1-2 nucleotides, changing the reading frame ○ In non-coding regions ▪ Promoter regions – may increase or decrease transcription ▪ Splice recognition sites – pre-mRNA may not be spliced correctly ▪ 5'UTR/3'UTR – alteration in ability of mRNA to be translated or in mRNA stability • Effects of a mutation on gene function ○ Null/amorph – produces no product ○ Hypomorph – produces a reduced amount or activity or